BIOCHEMICAL & MOLECULAR DEFENSE RESPONSES TRIGGERED BY ACIBENZOLAR S-METHYL AND A MULTIPLE-ELICITOR PLANT FORMULATION IN TOMATO

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Tese apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-graduação em Agronomia área de concentração em Fitopatologia, para a obtenção do título de "Doutora".

Orientador
Mário Lúcio Vilela de Resende, PhD

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LAVRAS
MINAS GERAIS - BRASIL

A minha querida mãe

e aos meus irmãos Fábio e Mara,

que incondicionalmente, me apoiaram sempre.

OFEREÇO

Ao meu filho Luís Henrique que me fez entender a razão da vida;

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GENERAL ABSTRACT

MEDEIROS, Fernanda Carvalho Lopes de. **Biochemical & molecular defense responses triggered by acibenzolar S-methyl and a multiple-elicitor plant formulation in tomato.** 2009. 135p. Thesis (Doctor in Phytopathology) – Federal University of Lavras, Lavras, MG.*

Undertaken major effort in plant biochemical research is to elucidate the defense signalling pathways in plants leading to resistance to plant pathogens and potentially triggered by elicitors. Hence, the analysis of gene expression profiles in response to an elicitor molecule treatment provided the basis to identify common and⁄or antagonistic features among defense pathways. To date, no data was published on the effect of natural formulations on genes expression profiles but earlier studies showed activation of PR-proteins and cell wall reinforcement as effective, suggesting that plant changes due to a natural formulation elicitor, might be involved in the induced defense. However, most previous work suggesting a defensive role for natural formulations provided data about downstream events (e.g., PR protein activity), without utilizing molecular techniques to reveal changes in gene expression levels. Our goal was to assess the role of a multiple-elicitor plant formulation based on coffee leaf (NEFID) in the induction of plant defense responses by probing defensive pathways at genomic and metabolic levels and comparing the responses to acibenzolar-S-methyl, a commercial inducer. In addition, we aimed to identify a set of candidate genes that would be regulated by the natural formulation and use these obtained results to better understand the molecular mechanisms underlying tomato induced resistance. For this purpose, tomato plants were treated with the elicitors, challenged or not with *Xanthomonas vesicatoria*, the disease severity, growth, leaf area were evaluated and the mechanisms activated were assessed, such as PR-1 and β-1,3- glucanase gene expression pattern, levels of PR proteins (chitinase and β-1,3-glucanase), lignin deposition, polyphenol-oxidase and peroxidase activities as well as the overall tomato gene expression using microarray approaches. Plantlets treated with NEFID demonstrated 35% disease severity reduction, a significatively reduction (61%) was obtained by ASM-treated plants. This severity reduction appears to be based on a rapid β-1,3-glucanase and PR-1 gene expression increase (12 hours after spraying) followed by an early response in a chitinase, glucanase and polyphenol oxidase activities (24 hours after spraying) and a high lignin deposition on leaf tissue. A total

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of 268 genes had changed regulation due to NEFID spraying, compared with water-treated control, with a majority of up-regulated transcripts which encoded mainly signal transduction, defense–related, oxidative burst and transcription factor genes. Since no evidence for salicylic acid or jasmonic acid buildup was found but mitogen activated proteins (MAP3K and MAPKK) as well as calcium dependent (calmodulin and phosphatidylinositol) signaling molecules were found, a SA-independent PR accumulation is likely to occur. The PRs chitinase, glucanase and peroxidase, with direct reported activity on pathogens, were up-regulated and they were not likely to suffer post-translational regulation since the corresponding enzyme activities were over-expressed as early as 24h after treatment and eventually remained as such for up to five days onward (glucanase and peroxidase). Therefore, the studied plant formulation represent a potential broad spectrum disease control.

Key-words: microarray, SAR, PR-1, chitinase, β-1,3-glucanase, peroxidase, RT-PCR, natural formulation, ASM

RESUMO GERAL

MEDEIROS, Fernanda Carvalho Lopes de. **Respostas de defesa bioquímicas e moleculares ativadas por Acibenzolar S-metil e por uma formulação natural a base de múltiplos eliciadores em tomateiro.** 2009. 135p. Tese (Doutorado em Fitopatologia) – Universidade Federal de Lavras, Lavras, MG.*

Esforços têm sido mobilizados para elucidar as rotas de defesa em plantas que levam à resistência a fitopatógenos, potencialmente ativadas pelo tratamento com eliciadores. Neste sentido, a análise da expressão de genes em resposta ao tratamento com moléculas eliciadoras representa uma ferramenta para identificar características comuns e/ou das rotas de defesa. Até o presente, nenhum dado foi publicado sobre o efeito de formulações naturais no perfil da expressão gênica, mas estudos anteriores mostraram a ativação de proteínas relacionadas à patogênese (PRs) e um potencial reforço da parede celular, sugerindo que as mudanças em plantas devidas a um eliciador, baseado em formulação natural, pode estar envolvido na defesa induzida. Contudo, as respostas raramente são correlacionadas a parâmetros de crescimento ou, feita uma abordagem multiplex para estudo não apenas de produtos de início de rota como aqueles de final. O objetivo deste trabalho foi avaliar o papel de uma formulação baseada em extrato de folha de café (NEFID) na indução de respostas de defesa de plantas, pelo monitoramento de rotas de defesa nos níveis genômicos e metabólicos e, compará-lo ao indutor comercial acibenzolar-S-metil. Objetivou-se também o estudo de genes candidatos regulados pela formulação natural (NEFID) para melhor entender os mecanismos moleculares envolvidos na resistência induzida de tomate. Para este fim, plantas tratadas com os eliciadores foram desafiadas ou não com *Xanthomonas vesicatoria* e avaliados a severidade da doença, o crescimento e área foliar bem como os mecanismos de ativação da resposta de defesa, avaliando-se o padrão de expressão de PR-1 e glucanase, os níveis de proteínas PR (quitinase e β- 1,3-glucanase), deposição de lignina, atividades de oxidase de polifenol e peroxidase assim como o perfil geral da expressão de genes de tomate usando a técnica do microarranjo. As plantas tratadas com NEFID demonstraram 35% de redução na severidade da doença e redução de 61% foi encontrada em plantas tratadas com ASM. Esta redução na severidade foi relacionada ao rápido aumento da ativação de β-1,3-glucanase e PR-1 (12 horas após tratamento) seguida por uma resposta rápida da atividade de quitinase, glucanase, polifenoloxidase (24 horas após tratamento) e deposição de lignina. Um total de 268 genes tiveram regulação

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mudada devido ao tratamento com NEFID, comparado com a testemunha tratada com água, com a maioria dos genes transcritos super-expressos, os quais codificaram principalmente para transdução de sinal, defesa, estresse oxidativo e fatores de transcrição. Uma vez que nenhuma evidência para o acúmulo de ácido salicílico ou jasmônico foi encontrada, mas proteínas quinases ativadas por mitogênos (MAP3K e MAPKK) assim como proteínas sinalizadoras dependentes de cálcio (calmodulina e fosfatidilinositol) foram encontradas, é provável que esteja ocorrendo um acúmulo de PR independente de ácido salicílico. As PRs, quitinase, glucanase e peroxidase, com atividade direta relatadas sobre patógenos, foram super-expressas e elas provavelmente não sofreram regulação pós-transcripcional, uma vez que a atividade destas enzimas foi super-expressa logo às 24h após o tratamento e eventualmente permaneceram assim por pelo menos mais cinco dias (glucanase e peroxidase). Portanto, a formulação a base do extrato de planta estudado representa um potencial para o controle de um amplo espectro de doenças.

Palavras-chave: microarranjo, SAR, PR-1, quitinase, β-1,3-glucanase, peroxidase, RT-PCR, formulação natural, ASM

CHAPTER 1

1 GENERAL INTRODUCTION

Tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Miller) is an economically important crop worldwide, and a preeminent model system for genetic studies in plants (Barone et al., 2007). Bacterial leaf spot is caused by the bacterium *Xanthomonas vesicatoria* in tomato (Lycopersicon esculentum) and infection results in a decreased yield at harvest. Control of the disease is difficult, often requiring expensive and complex integrated pest management (IPM), including the use of contamination-free seeds, sanitization practices, and the use of chemicals (Araújo et al., 2003).

Plants are able to survive an attack by a potential pathogen when prior treated with elicitors of defense responses, reacting with a local and systemic induction of a succession of defenses that prevent or contain the infection and provide enhanced resistance to subsequent infections by the same or even unrelated pathogens (Montesano et al., 2003). Plants can also induce defense reactions to a broad range of pathogens as a result of prior exposure to pathogens or physical stress (Metraux, 2001). The ability of plants to react to an invader by triggering local and systemic responses was explained by the production of a signal released from the infected leaf and translocated to other parts of the plant where it induces defense reactions, known as systemic acquired resistance (SAR). SAR underlines the ability of plants to acquire a state of general resistance after an initial infection (Metraux, 2001).

However some plant extracts can be made up of multiple elicitors. An natural formulation from coffee leaves (NEFID) is likely to have multiple plant and microbe-derived elicitors and has proven to be effective against multiple pathogens. The natural formulation protected tomato plants against bacterial

pathogen. Cotton plants sprayed with NEFID had a reduction in the severity of bacterial blight and this result was similar to ASM (Ishida et al., 2007)The product has also been effective for the control of coffee rust and phoma spot (Barguil et al., 2005; Santos et al., 2007).

To evaluate the role of a natural formulation based on *Coffea arabica* leaves or acibenzolar-S-methyl in triggering induced resistance, tomato plants were treated with the elicitors, challenged or not with *Xanthomonas vesicatoria* and the mechanisms activated were evaluated, such as levels of PR proteins (chitinase and β-1,3-glucanase), lignin deposition, polyphenol-oxidase and peroxidase activities as well as the overall tomato gene expression using microarray approaches.

2 LITERATURE REVIEW

This chapter presents an overview of defense responses and the microarray approach to identify a set of candidates genes to better understand the molecular and biochemical mechanisms underlying induced resistance.

2.1 Aspects of induced resistance to pathogens

In nature, plants are continuously exposed to pathogens and the preventive elicitation for resistance helps those facing further attacks. Plants respond to pathogens by activating a variety of defense mechanisms. These defense responses include hypersensitive programmed cell death (Dangl et al., 1996; Greenberg, 1997), induction of defense or defense-related genes (Dixon & Harrison, 1990), cross-linking and reinforcement of cell walls (Brisson et al., 1994), biosynthesis of phytoalexins and metabolism of phenolic compounds (Nicholson & Hammerschmidt, 1992), and production of active oxygen species, such as singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radical (Lamb & Dixon, 1997; Bolwell, 1999). A hypersensitive response at the site of infection is often manifested as necrotic lesions resulting from host cell death (Staskawicz et al., 1995; Dempsey et al., 1999). In the distal uninfected parts of the plant, systemic acquired resistance develops to provide long-lasting broad spectrum resistance against pathogens.

2.2 The mechanisms involved in systemic acquired resistance

2.2.1 Enzymes involved in the induction of resistance:

a. Peroxidases

A class III plant peroxidase (POX, EC 1.11.1.7) is a glycoprotein that catalyzes oxidoreduction between H_2O_2 and various reductants (Hiraga et al., 2000).

Many reports have suggested that POXs play roles in resistance to pathogens such as lignification and suberization (Dean & Kolattukudy, 1976, Quiroga et al., 2000), cross-linking of cell wall proteins (Showalter, 1993), xylem wall thickening (Hilaire et al., 2001), generation of reactive oxygen species (Bestwick et al., 1998), hydrogen peroxide scavenging (Kawaoka et al., 2003), phytoalexin synthesis (Kristensen et al., 1999), antifungal activity of POX itself (Caruso et al., 2001) and auxin metabolism (Lagrimini et al., 1997). However, it is difficult to define the specific functions of individual POXs because of their low substrate specificity in vitro and the presence of many isoenzymes (Hiraga et al., 2000).

Some *pox* genes are activated by infection with pathogens such as fungi (Thordal-Christensen et al., 1992, Harrison et al., 1995, Curtis et al., 1997), bacteria (Young et al., 1995, Bestwick et al., 1998), viruses (Lagrimini and Rothstein 1987, Hiraga et al., 2000b) and viroids (Vera et al., 1993). Therefore, it is classified to the pathogenesis-related (PR) protein-9 family (van Loon et al., 1994).

Many studies have indicated the importance of POXs in defense against pathogen infection. On tomato plants infected with *Xanthomonas vesicatoria*, POX activity accumulated when plants were elicited with a plant formulation or the commercial product acibenzolar-S-methyl as early as 4h after treatment and its expression remained steady until 72h after treatment (AT), the last sampled time point (Cavalcanti et al., 2006). The enzyme was also reported as part of the resistance against fungal infection. Plants treated with 2,6-dichloroisonicotinic acid (INA) and challenged with powdery mildew accumulated peroxidase in a fast manner until 3 days AT and kept it steadily up

until 15 days AT. The induction of the scavenger enzyme was accompanied by an increase in β-1,3-glucanase and, plants treated with the elicitor and subsequently treated with diphenylene iodinium, an inhibitor of the oxidative burst, had a reduced level of not only POX but also β-1,3-glucanase activities. The same authors observed that plants treated with H_20_2 expressed high levels of the scavenger but no expression of the PR, suggesting that the peroxidase induced upon elicitation is part of a defense network to hinder the pathogen development (Kang, 2009).

b. Polyphenol oxidases

Polyphenol oxidases (PPOs; EC 1.14.18.1 or EC 1.10.3.2) are nuclear-encoded enzymes of almost ubiquitous distribution in plants (Mayer & Harel, 1979; Mayer, 1987). PPOs catalyze the oxygen-dependent oxidation of phenols to quinones.

Because of their conspicuous reaction products and their wound and pathogen inducibilities, PPOs have been suggested to participate in plant defense against pests and pathogens (Mayer & Harel, 1979; Mayer, 1987; Steffens et al., 1994; Constabel et al., 1995; Thipyapong et al., 1995; Thipyapong & Steffens, 1997). Systemic induction of PPO expression in response to wounding and pathogens might provide an additional line of defense to protect plants against further attack by pathogen and insects (Bashan et al., 1987; Constabel et al., 1995; Thipyapong et al., 1995; Stout et al., 1999).

The activity of PPO is also of broad spectrum, acting in the control of fungal and bacterial pathogens (Cavalcanti et al., 2007; Daw et al., 2008). The accumulation of polyphenol oxidase was associated with peach resistance to decay when treated with methyl jasmonate, a well-known plant defense elicitor (Jin et al., 2009). The PPO activity has also been reported in plants treated with

natural formulations also showing disease resistance (Cavalcanti et al., 2007; Daw et al., 2008).

Down regulation of all the members of the PPO gene family was observed when antisense PPO cDNA was introduced in tomato plants and examined the resistance of the plants to the pathogen *Pseudomonas syringae*. PPO activity was reduced by a factor of about 40. Examination of the sensitivity of the plants to the pathogen revealed a dramatic increase in their susceptibility, although the overall growth and development of the tomato plants was not affected by the down regulation of PPO (Thipyapong et al., 2004).

In other experiment in which PPO was over-expressed in tomato plants (Li & Steffens, 2002), the over-expression was accompanied by enhanced resistance to the same pathogen (*P. syringae*). The levels of mRNA rose to a much greater extent than the levels of PPO protein. These findings clearly implicate PPO in the defense of plants against pathogens but do not as yet provide an explanation of the underlying mechanism.

c. Glucanases

The glucanases (β-1,3-endoglucanases; EC 3.2.1.6) are present in a wide variety of plants, animals and microorganisms (Jwanny et al., 2001).

Plant β-1,3-glucanases are referred as PR-2 proteins and are subdivided into three classes. Class I glucanases are basic proteins of about 33 kDa and are localized in the plant vacuole (Bulcke et al., 1989). Classes II and III include acidic, extracellular proteins of about 36 kDa (Theis and Stahl, 2004). They participate in several physiological and developmental plant processes. In addition, class I β-1,3-glucanases exhibit antifungal activity both *in vitro* and *in planta* (Joshi et al., 1998; Mauch et al., 1988). Class II β-1,3-

glucanases exhibit *in vitro* antifungal activity only if applied in combination with chitinases or class I β-1,3-glucanases (Theis and Stahl, 2004).

d. Chitinases

Chitinases (EC 3.2.1.14) constitute the second largest group of antifungal proteins. They catalyse the hydrolytic cleavage of the β-1,4-glycoside bond present in biopolymers of N-acetyl-D-glucosamine producing chito-oligosaccharides of 2–6 N-acetyl-D-glucosamine residues in length (Stintzi et al., 1993).

Chitinases are classified in families 18 and 19 of the 57 families in which O-glycoside hydrolases are presently subdivided (Henrissat and Bairoch, 1996). Higher plants synthesize seven different classes of chitinases which differ in protein structure, substrate specificity, mechanism of catalysis and sensitivity to inhibitors (Brunner et al., 1998). These classes are grouped into three families of PR proteins (Neuhaus et al., 1996): chitinases of classes Ia, Ib, II, IV, VI and VII belong to the PR-3 family, whereas those of classes III and V are included in the PR-8 and PR-11 families, respectively. Additionally, some proteins with low endochitinase activity occur in the PR-4 family (chitin-binding proteins) (Melchers et al., 1994). Acidic chitinases belonging to classes Ib, II, III, IV and VI are secreted to the apoplast, whereas basic chitinases included in classes Ia, III and VI are located in vacuoles (Arie et al., 2000).

Chitinases have been reported to play a role in growth and development such as vegetative storage protein (Peumans et al., 2002), antifreeze activity (Yeh et al., 2000), aspartic protease and α-amylase inhibitor activity (Ary et al., 1989; Guevara et al., 1999) but its more widely reported function is on disease resistance.

Plant chitinases that hydrolyze chitin, inhibit the growth of fungi and generate chitin oligosaccharides that act as new elicitors for long-term

resistance induction (Sharp et al., 1984). In addition, many chitinases are induced by pathogen attack and some isoforms exhibit *in vitro* antifungal properties. For these reasons, chitinases are believed to play a major role in plant host defense against pathogens.

The antifungal activity displayed by many chitinases was initially assumed to derive from their ability to digest chitin, leading to a weakened fungal cell wall and subsequent cell lysis. However, recent evidence indicates that the mechanisms by which chitinases inhibit fungal growth seem to be more dependent on the presence of a chitin-binding domain than on chitinolytic activity. Thus, the antifungal activity of a tobacco class I chitinase is three times higher when a chitin-binding domain is present (Iseli et al., 1993).

Not rarely the activities of glucanases and chitinaes have been reported as being operative in induced plants against pathogens (Campos et al., 2009; Cavalcanti et al., 2006) and the elicitation may be accomplished by a broad range of products, from commercial resistance inducers to plant formulations and avirulent pathogens (Campos et al., 2009; Cavalcanti et al., 2006).

An increase of up to eight fold in levels of both glucanase and chitinase were observed when non-pathogenic *Colletotrichum lindemuthianum* race Delta strains were used to treat common bean plants and the increase in the detected enzyme highly correlated with the control of anthracnose (Campos et al., 2009). The enzymes were also reported to be over-expressed in tomato plants when treated with plant resistance activators and later challenged with a bacterial pathogen (Cavalcanti et al., 2006). Both glucanases and chitinases have lisozyme activity in the hydrolysis of the β(1-4) bounds between N-acetyl glucosaminic and muramic, which is components of the bacterial cell wall (Majeau et al., 1990).

Since the majority of pathogens are susceptible to glucanases and chitinases, plants over-expressing these pathogenesis-related proteins represent a plausible strategy to assure disease control (Honée, 1999). Furthermore, the degradation of fungal cell wall releases oligomers that are recognized by the plant and triggers broad spectrum disease resistance genes (Buchannan, 2000) effective not only against fungal pathogens but also against virus (Guo et al., 2002).

Some plants are particularly susceptible to a wide range of pathogens and its production is highly dependent on fungicide spray-based disease control. One of such is tomato, the plant has been bred for some diseases but the constitutive broad spectrum disease control has yet to be achieved. Therefore, Tabaeizadeh et al., (1999) constructed transgenic tomato lines over-expressing chitinase and glucanase from its wild relative *Lycopersicon chilense*. As a result of the engineering, plants became more resistant to vascular wilts.

However, the broad spectrum disease control by the over-expression of PR-proteins may not be an ecological acceptable technology, since transgenic plants may harm non-pathogenic fungi, some of which have a direct beneficial effect on plant growth such as mycorrhizae in increase the phosphorus up-take (Girlanda et al., 2008). Furthermore, both chitinase (Lutz et al., 2003) and glucanase (Damasceno et al., 2008) have already been reported as present among plant pathogenic fungi and by exerting a selection pressure by the only use of transgenic plants expressing those genes may lead to failure of the disease control strategy.

On the other side, glucanse and chitinase expression after exogenous elicitor treatment is generally accompanied by the induction of a series of other disease control responses such as cell wall cross-linking, lignifications, production of phenolics as mentioned in the other sections.

2.2.2 Lignification and Other Structural Barriers

Lignin is one of the most structurally complex of the biopolymers. The chemical construction of lignin a three-dimensional, branched polymer formed by the oxidative polymerization of three substituted cinnamyl alcohols: p-coumaryl alcohol (I), coniferyl alcohol (II), and sinapyl alcohol (III).

Regardless of the nature of the formed lignin in plants, evidence suggests that the esterification of polymerized phenols to cell-wall materials is strongly related to the expression of resistance (Fry, 1986; Fry, 1987). Since it accumulates rapidly following infection (Farmer, 1985, Grand et al., 1987) and has been implicated in the chemical modification of cell walls to be more resistant to its degrading enzymes, increase in the resistance of walls to the diffusion of toxins from the pathogen to the host and of nutrients from the host to the pathogen, produce toxic precursors and free radicals, and the actual lignification and entrapment of the pathogen (Ride, 1978). In fungal penetration sites, lignin is deposited to hinder the pathogen development in structural barriers such as papillae (Cadena-Gomez & Nicholson, 1987; Ride & Pearce, 1979; Vance et al., 1980).

Hence, the role of pathogen-induced lignin and related polymers has been closely correlated with the defense responses in several plants to multiple pathogens (Vance et al., 1980).

The accumulation of lignin was related to the control of soybean to *Sclerotinia sclerotiorum* in resistant cultivars (Peltier et al., 2009). This accumulation was also observed in the resistance of cotton plants to root rot pathogens.

For foliar pathogens the lignin content as well as total phenols have also been correlated with disease resistance. Reimers & Leach (1991) have

shown that the race-specific resistance of rice carrying the *Xa-lO* gene to *X. oryzae* is correlated with the deposition of lignin at the site of infection. A similar finding was observed for tomato cultivars resistant to *Xanthomonas vesicatoria* (Kavitha & Umesha, 2008).

2.3 Elicitors for systemic acquired resistance

Several elicitors have been studied as triggers of systemic acquired resistance (SAR). Vallad & Goodman (2004) reviewed the commercially available ones and the obtained field results. For most of the results where it was compared to a standard control there was no observed statistical difference.

In their review the more commonly cited product was acibenzolar-S-methyl (Bion or Actiguard as trade names) a commercial product derived from benzothiadiazole (BTH), which in turn is a functional analog of salicylic acid, known to stimulate the production of plant defense-related compounds and induce systemic acquired resistance (SAR), distinguished from other plant defense responses by local and systemic activation of specific pathogenesis-related genes (PR), mentioned in detail in the previous sessions (Thaler et al., 1999; Kuc, 2001; Durrant & Dong, 2004).

Although ASM has proven to be effective against a wide range of pathogens and pests, it is particularly an attractive tool for the control of bacterial and virus disease, where effective control is hardly achieved.

Herman & Smart (2007) studied the expression level of the SAR marker gene in tomato following ASM sprays in three cultivars. While early expression of PR-1 (a marker gene of SAR) increased by up to 5 fold until 3 days after spraying (DAS), this increase was dramatically different among cultivars and peaked at different time-courses. The cultivar Rio Grande peaked 22 fold increase early in the time-course (7-8 DAS) but dropped sharply afterwards, Rutgers cultivar peaked later 9-10 DAS and dropped more

smoothly and Supersonic cultivar had a more flat bell-shaped curve similar to the one obtained in the early studied time-course.

The plant disease resistance activator does not assure 100% protection against bacterial spot of tomato in the field but is an important player in the integrated pest management. Robertsa et al., (2008) obtained 12% disease control when using ASM alone but achieved up to 67% when alternating ASM sprays with copper hydroxide and mancozeb.

Many other commercial disease resistance inducers have been studied with variable efficacy (reviewed by Resende et al., 2006). A promising field of study is the prospection of leaf formulations with the potential for SAR triggering.

A suspension of *Crinipellis perniciosa* mycelium protected tomato plants against *Xanthomonas vesicatoria* by up to 87% and the main elicitor part of the preparation was mainly made up of heterologous chitosan (Cavalcanti et al., 2007).

However some plant extracts can be made up of multiple elicitors. A seaweed based extract has laminarin and carrageenans, two polysaccharides which are recognized by inespecific receptors on the plant and triggers defense related genes in tobacco (Mercier et al., 2001). Another formulation from coffee leaves (EFID) has recently been patented (I.N.P.I., Protocol number 0000220604167501, "Formulação para indução de resistência"... Aug/2006) has not been fractionated but is likely to have multiple plant and microbe-derived elicitors and has proven to be effective against multiple pathogens. The product has been effective for the control of coffee rust and phoma spot and one possible mechanisms of resistance is the buildup of lignin content in the leaves following plant sprays (Barguil et al., 2005; Santos et al., 2007).

The plant formulation also protected tomato plants against bacterial pathogen. Cotton plants sprayed with EFID had a reduction in the severity of bacterial blight and this result was similar to ASM (Ishida et al., 2007).

2.4 Functional genomics in the study of plant defense against pathogens

When a plant is treated with a plant defense activator, several responses have been elucidated but the overall changes in the plant are starting to be investigated and one important tool being used is the microarray technique.

In order to better understand the operation of the SAR pathway, using *Arabidopsis thaliana* for which a vast literature has already been generated, the genome has been fully sequenced and most genes are known, Blanco et al. (2009) identified two pathways for the activation of SAR genes could be distinguished, the NPR1 independent represent only 14% of them. These two groups of genes, not only differ in their main functional categories, but also in their timing and mechanism of activation by SA. Not only have been found defense related responses but SAR elicited plants also displayed genes involved acclimatory responses to stress, such as recovery of the cell redox balance (glutathione transferase, UDP glycosyl transferase and glutaredoxins), intracellular stress signaling, improvement of pathogen recognition, and promotion of metabolic changes. Glutaredoxins along with thioredoxins catalyze reversible thiol-based reduction of target proteins, implicated in the SA-dependent reduction of NPR1 and TGA factors required for the transcription of defense genes and ecotopic expression of a glutaredoxin gene silenced the expression of PDF1.2 suggesting that this gene may be one of the switches in the induced systemic resistance pathway (SAR/ISR).

Since early studies on SAR-associated events, Maleck et al. (2000) identified not only transcripts peculiar to this pathway but also co-regulated traits involved in redox homeostasis.

Even if the whole genome has not yet been sequenced for all plant species, many genes have known functions and several attempts are helping to better understand how commercial plants defend themselves against pathogens.

An over 33,000 citrus gene chip is available and helped Kim et al. (2009) explain what changes *Candidatus liberibacter* has on the plant in an attempt to explain the plant pathogen interactions. They found that the clogged sieved elements are made up mainly of carbohydrates and this corroborates with the leaf up-regulation of three key starch biosynthetic genes including ADP-glucose pyrophosphorylase, starch synthase, granule-bound starch synthase and starch debranching enzyme.

In a 7,883 microarray chip, customized for ESTs of rust infected soybean at different time points and deposited sequences, cultivars carrying the genes Rpp1-Rpp4 conferring resistance to *Phakopsora pachyrhizi,* 558 were found to have changed regulation among which the defense related ones represented to highest group and they mainly belonged either to peroxidases or lipoxygenases. Conversely, cell-wall-associated protein such as extensins, proline-rich proteins, and xyloglucan endotransglycosylases were found to be down-regulated (Choi et al., 2008).

Similar studies on potato gene response from cultivars resistant to late blight (*Phytophtora infestans*) using a customized cDNA chip carrying 1,009 spots, defense-related metabolic pathways such as the biosynthesis of aromatic amino acids, phenylpropanoids, oxylipin, and ethylene, were activated, which resulted in the *de novo* synthesis of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), suggesting that a combination of both reported signaling

pathways (SAR and JA) may be involved in the defense of potato cultivars to late blight.

Another Solanaceous species, with a simpler genome organization is tomato (*Solanum lycopersicon* L.). It is diploid, with a short generation time, routine transformation technology, and availability of rich genetic and genomic resources. It has a diploid genome with 12 chromosome pairs and an estimated genome size of 950Mb encoding approximately 35,000 genes that are largely sequestered in contiguous euchromatic regions (reviewed by Barone et al., 2008). Several microarray gene chips are marketed and presently cover up to 1/3rd of the estimated size of genome (Van der Hoeven et al., 2002). The great advantage of tomato as a model system to study metabolic changes is the vaste number of genus relatives it covers (potato, eggplant, tobacco and pepper) (Moore et al., 2005).

Tomato plants were challenged with fusicoccin (FC), a diterpene glucoside toxin produced by the fungus *Fusicoccum amygdali* Del. to observe defense-related genes. Pronounced changes in transcript abundance of pathogenesis-related and a conversely down-regulation of salicylic acid synthesis suggest for an induction of PRs SA-independent. The defense responses are not JA-dependent either since a down-regulation of wound responsive genes was observed. Conversely, the jasmonic acid synthesis was found to be up-regulated and this has been linked to the JA ability inhibit the biosynthesis of photosynthetic pigments and photosynthetic activity which corroborates with the predominant down-regulation of photosynthesis related genes. The SA-independent induction of PRs was assumed to occur via mithogen activated kinase and through Ca^{2+}-dependent calmodulin (Frick & Schaller, 2002).

In order to control post-harvest diseases, *Cryptococcus laurentii* a biocontrol yeast was used to spray cherry tomato fruits and using microarray

technology, a changed regulation of 531 genes was observed. Defense-related genes related to the SAR-dependent PR activation (chitinase and glucanase) were observed and an already shown interplay of salicylic acid and jasmonic acid/ethylene defense was present. Interestingly, a down-regulation of ethylene synthesis has direct implications on the fast ripening and susceptibility to disease. Other defense related responses involved the up-regulation of cytochrome P450 and a down-regulation of cell wall loosening and expansion (expansin, xyloglucan endoglycosl transferase, polygalacturonase), enzymes directly related to fungal development (Jiang et al., 2009).

To date, no data was found on the effect of plant formulations on plant global changes but earlier work (Medeiros, unpublished) showed activation of PRs and cell wall reinforcement is operative suggesting plant changes due to a plant formulation elicitor might be involved in the broad spectrum defense. Hence, the present work will probe the responses to a multiple-elicitor plant formulation based on coffee leaf on tomato.

3 SPECIFIC OBJECTIVES

Our goal was to assess the role of Acibenzolar S-Methyl and a multiple-elicitor plant formulation based on coffee leaf (NEFID) in the induction of plant defense responses by probing defensive pathways at genomic and metabolic levels. Most previous work suggesting a defensive role for natural formulations such as NEFID provided data about downstream events (e.g., PR protein activity), without utilizing molecular techniques to reveal changes in gene expression levels, no data was found on the effect of natural formulation on plant global changes. In addition, we aimed to identify a set of candidates genes that are regulated by the natural formulation and use these results obtained to better understand the molecular mechanisms underlying tomato induced resistance. Owing to the possessions of knowledge of plant-bacterial interactions in tomato, this plant system are likely to provide good models for assessing the role of these products in triggering defense responses. Monitored defense responses include: severity of black spot disease after treatments with the tested formulations, levels of PR proteins (chitinase and β-1,3-glucanase), lignin deposition, polyphenol-oxidase and peroxidase activities as well as the overall tomato gene expression using microarray approaches.

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CHAPTER 2

Induction of pathogenesis-related proteins and resistance by a natural formulation of coffea leaf and acibenzolar-S-methyl in tomato seedlings against bacterial spot.

ABSTRACT

The efficiency of a natural formulation of coffee leaf (NEFID) and the plant defense inducing compound, acibenzolar S-methyl (ASM) was tested, on tomato seedling growth and on induced defense response against bacterial spot. Plantlets were sprayed with either NEFID or ASM and subsequently challenged with a virulent strain of *Xanthomonas vesicatoria* 3 days after. Disease severity, plant growth and leaf area were evaluated. To understand defense related responses, PR-1 and β-1,3-glucanase gene expression patterns were evaluated as well as the induction of β-1,3-glucanase (GLU), chitinase (CHI), polyphenol oxidase (PPO) activity, and lignin deposition, were compared in treated and control plants. NEFID and ASM treated plants demonstrated a reduced disease severity by 35 and 61% respectively This reduction in disease severity was correlated with increases in β-1,3-glucanase and PR-1 gene expression 12 hours after spraying and elevated chitinase, glucanase and polyphenol oxidase activity 24 hours after spraying; higher leaf lignin deposition was also observed at 10 and 23 days after spraying.

Keywords: systemic acquired resistance, coffea natural formulation, PR-1, chitinase, glucanase, polyphenol oxidase, lignin

RESUMO

A eficiência de uma formulação natural a base de folha de café (NEFID) e do composto comercial indutor de resistencia em plantas acibenzolar S-metil (ASM) foi testada no crescimento de mudas de tomateiro e na resposta de defesa induzida contra a mancha bacteriana. Plântulas foram pulverizadas com NEFID ou ASM e subsequentemente inoculadas com uma estirpe virulenta de *Xanthomonas vesicatoria*, três dias após o tratamento. Foram avaliados a severidade da doença, o crescimento da planta e a área foliar. Para melhor entender os mecanismos de resposta de defesa da planta, padrões de expressão dos genes PR-1 e β-1,3-glucanase foram também avaliados assim como, a atividade das enzimas β-1,3-glucanase (GLU), quitinase (CHI), polifenol oxidase (PPO) e deposição de lignina foram comparados em plantas tratadas e controle. Plantas pulverizadas com NEFID e ASM demonstraram redução da severidade da doença em 35 e 61% respectivamente. Esta redução na severidade da doença foi correlacionada com o aumento na expressão dos genes β-1,3-glucanase e PR-1, 12 horas após a pulverização e, a uma elevada atividade de quitinase, glucanase e polifenol oxidase 24 horas após a pulverização; uma maior deposição de lignina nas folhas foi também observada aos 10 e 23 dias após a pulverização.

Palavras-chave: Resistência sistêmica adiquirida, formulação natural a base de café, PR-1, quitinase, glucanase, polifenol oxidase, lignina

1 INTRODUCTION

The bacterial spot (*Xanthomonas vesicatoria* Doidge) is a serious seed-borne tomato disease efficiently seed-transmitted. Carmo et al. (1996) showed that only one seed out of 10,000 in a seed-lot is a sufficient inoculum for a 100% bacterial spot incidence in bell pepper under favorable environmental conditions, such as the one that prevails under tropical growing regions (Al-Dahmani et al., 2003).

While seed testing does not eliminate the possible seed-lot contamination and efficient seed treatment still has detrimental consequences on germination (Carmo et al., 2004), plant protection strategies can improve the health and productivity of tomato plants

The benzothiadiazole derivative benzo(1,2,3)thiazole-7carbothioic acid-S-methyl ester (acibenzolar- S-methyl, ASM, or BTH) has been developed as a potent systemic acquired resistance (SAR) activator that does not have antimicrobial properties, but instead increases crop resistance to disease by activating the SAR signal transduction pathway in several plant species including tomato (Achuo et al., 2004; Bokshi et al., 2003; Lawton et al., 1996; Soylu et al., 2003). The use of acibenzolar-S-methyl protected plants by 47.7% compared to the water pre-treated control and the well-known mechanism is the salicylic acid resistance (SAR) induction by the activation of defense-related enzymes peroxidase and polyphenol oxidase starting within hours after spraying (Cavalcanti et al., 2006).

The SAR is also associated with the induction of chitinases, β-1,3-glucanases and other pathogenesis-related (PR) proteins that can hamper not only the development of bacterial pathogens but also fungal, nematodal and even viral-associated plant diseases (Van Loon & Van Strien, 1999) but also make it difficult for pathogens to develop resistance (Hammond-Kosack &

Parker, 2003). Induction of plant defenses against pathogens also involves physical barriers such as cell wall reinforcement via lignin deposition (Anterola & Lewis, 2002; Thangavelu et al., 2003) resulting of an increase in enzyme activities, such as phenylalanine ammonia-lyase and polyphenol oxidases, related to the phenylpropanoid pathway (He et al., 2002). Similar activation of SAR is observed after exposition of plants to microbial surface-derived molecules such as peptides, carbohydrates, glycoproteins and lipids or plant derived cell wall (Nürnberger & Brunner, 2002). An infected coffee leaf formulation EFID combines both fungal and plant derived elicitors and has efficiently controlled coffee rust under field conditions and induced the accumulation of lignin compared to the water pre-treated control (Santos et al., 2007).

The aim of this research was to compare the effectiveness of a natural formulation of coffee leaf NEFID and that of a known plant defense inducing compound, – ASM, on tomato seedling growth and on resistance induction against *X. vesicatoria* inoculation. The present study was also aimed at understanding the defense related response by comparing the expression pattern of selected defense related mRNAs and the induction of the corresponding proteins after treatment with chemical and biological elicitors.

2 MATERIAL AND METHODS

2.1 Plant material and inoculum preparation

For the biochemical determinations and the disease severity measurement tomato seeds (*Solanum lycopersicum* var. Santa Cruz Kada) susceptible to *X. vesicatoria*, purchased from Isla Sementes Ltda (Porto Alegre — Rio Grande do Sul, Brazil) were surface sterilized in 1% (v/v) ethanol for 3 min, followed by 1.0 g L^{-1} sodium hypochlorite for 1 min. After thorough rinsing with distilled water they were planted in 3 L pots filled with Plantmax® substrate in a greenhouse at a mean temperature cycle of 28±3°C day/23±3°C night, relative humidity of 40±3%/85±3%, and a 12 h photoperiod, at 450–500 $mmolm^{-2}$ s^{-1} maximum photon flux densities, measured at plant level (IRGA, model LCA-4, Hoddesdon, UK). For analysis by reverse transcription–polymerase chain reaction (RT–PCR) experiments were conducted in a growth chamber in which temperature was maintained at 29±4°C with a relative humidity 40±10%. Plants were grown under metal halide and high pressure sodium lamps for a 16-h/8-h light/dark photoperiod with a total light intensity of 700 $mmol/m^2/s$.

Twenty-five days after planting, when seedlings were 20±4 cm tall, the tomato leaves were sprayed with the test substances in order to determine their capacity to protect themselves from bacterial spot.

Bacterial cultures of *Xanthomonas vesicatoria* strain 89-T from the Embrapa's (Centro Nacional de Pesquisa de Hortaliças) culture collection were cultivated at 28°C in a culture media Kado & Heskett 523. Inoculum was obtained from bacterial cells during the log-phase growth period, cultured in a liquid Kado & Heskett 523 medium and incubated under shaking at 200 rpm at 28°C for 12 h (dark). The bacterial cells were concentrated by centrifugation (twice each at 3000g, 5 min) and resuspended in sterile distilled water.

Inoculum concentration was adjusted by dilution with sterile distilled water to give an absorbance of 0.200 at 540 nm, corresponding to 10^{11} cfu L^{-1}. For long-term storage, bacterial cultures were maintained at -80°C in in a liquid Kado & Heskett 523 medium that contained 20% (v/v) glycerol.

2.2 NEFID and ASM

NEFID formulation which has as its main raw material coffee leaves (*Coffea arabica*), collected from field soil surface (due to disease, harvest fruit and/or other stresses) and selected for powder production. A hundred grams of this powder is mixed with 1000 mL distilled water, boiled in reflux and filtered through a sieve of 400 meshes. The filtrate of leaves is sampled and stored at -20^{o}C. The formulation based on NEFID is patented by Resende et al. (2006) **(I.N.P.I., Protocolo número 0000220604167501, FORMULAÇÃO PARA INDUÇÃO DE RESISTÊNCIA... 02 de Agosto de 2006).**

Bion® [acibenzolar-S-methyl or benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester] (ASM) purchased from Syngenta Proteção de Cultivos Ltda in the form of a 500 g kg^{-1} dry powder active ingredient (ASM) and used as a 0.2 g ASM L^{-1} aqueous solution.

2.3 Treatment, assessment of disease severity and preparation of samples for determining gene expression and enzyme activities.

Intended for disease severity, tomato plants at 25 days after planting were uniformly sprayed on the aerial portion of the plant with ASM, NEFID or distilled water (control) until runoff. Three days after treatment the bacterial inoculation was performed, spraying 80 mL of *Xanthomonas vesicatoria* cell suspension (10^{11} cfu per L^{-1}) until runoff, in control and treated inoculated tomato plants. Disease symptoms were evaluated at seven days after bacterial inoculation. The severity of bacterial spot was assessed by visual diagrammatic scale rating of lesions on a 1-50% (the percentage of lesions present on the total

leaf area) (Mello et al., 1997). Leaf area was evaluated at the end of the trial period 20 days after spraying, using ImageJ software. Seedlings heights were evaluated at 3, 10, 13, 16 and 20 days after spraying.

Designed for analysis by reverse transcription–polymerase chain reaction (RT–PCR), plants were harvested at different time points (12, 24, 48, 72, 96, 144 h) after the treatment spraying. All plant materials were harvested during the light period at 1:00 p.m. to avoid light/dark effects on gene expression. Individual plants were used for each time-point harvest.

For enzymatic assays, 25 day-old tomato plants were sprayed with ASM, NEFID or distilled water (control) until runoff. Treated and control plants were harvested at 0, 24, 48, 72, 96, 120 and 240 hours after sprayings (HAS). In an additional trial, 25 day-old plants were sprayed with the test substances and three days later challenged by inoculation with 80 mL of *X. vesicatoria* cell suspension (10^{11} cfu l^{-1}). The excised leaves were frozen in liquid nitrogen and immediately utilized.

2.4 Reverse transcriptase-mediated PCR

Total leaf RNA was isolated with an RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Five micrograms of RNA was reverse-transcribed into cDNA using 2µL MuMLV-RT enzyme (Promega, Madison, WI, USA). Each reaction mixture contained 5 µg of RNA, 4µL oligo dT, 4 µL dNTPs (2.5 M), 1µL RNasin, 6µL DEPC water and 10µL of 5X MuMLV buffer, and samples were incubated at 37^{o} C for 1 h. For the determination of transcript quantities, the first strand cDNA was amplified in a PCR reaction using gene-specific primers:

- *pr-1*: 5'-ACTCAAGTAGTCTGGCGCAACTCA-3' and 5'-AGTAAGGACGTTGTCCGATC CAGT-3' - *Lycopersicon esculentum*

pathogenesis-related protein (PR1) mRNA gi|76363946| gb|DQ159948.1| with a product size of 124bp.

- *glu*: 5'-AAGCAATCGGTGAAGCTGGTTTGG-3' and 5'-ATGGCCATCCACTCTCTGAC ACAA-3', *Lycopersicon esculentum* beta-1,3-glucanase mRNA, gi|170381|gb|M80608.1| TOMB13GLUB, with a product size of 381bp.

- *act*: 5'-TTGACTGAGGCACCACTTAACCCT-3' and 5'-GCTTTCAGGTGGTGCAACGACTTT-3', *Lycopersicon esculentum* actin gene, gi|1498360|gb|U60478.1|SLU60478, with a product size of 777bp.

The PCR volume was 50µl, containing 100 ng of each primer, 4µl dNTPs, 5µl of cDNA, and 0.5 units of Taq DNA polymerase (Fisher Scientific, USA). A Programmable Thermal Controller (MJ Research, Watertown, MA, USA) was used for the amplification of *pr-1, glu, act* cDNAs, using 25 cycles, under optimum dynamic ranges before reaching the plateau. Equal amounts of PCR products were separated by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide. *Lycopersicon esculentum* actin, was used to ensure equal loading of the lanes. RT–PCR analysis was performed at least in triplicate. For both experiments, agarose gel electrophoresis images were taken by Kodak Gel Logic 100 Imaging System (Fisher Scientific, Houston, TX, USA) and the band intensity quantified by Image J 1.33u (http://rsb.info.nih.gov/ij/, National Institute of Health, USA). Normalization of signal was based on the housekeeping gene *Solanum lycopersicum* actin (NCBI gi|1498360|).

2.5 Enzyme extraction, PR-protein, defense enzyme assay

Treated and control, non-inoculated collected at 0, 24, 48, 72, 96, 144 and 240 hours after spraying (HAS) and inoculated with *X. vesicatoria* assemble at 72, 96, 144 and 240 HAS, tomato fresh leaf (1.0 g) was

homogenized for 5 min in a mortar with a pestle in 3 mL of ice-cold 50mM sodium acetate buffer pH 5.2, containing 0.1mM EDTA. After filtration, the homogenate was centrifuged at 13,000g for 15 min and the supernatant (crude extract) used as the source of enzymes. All the steps were carried out at 0–4^{o} C (Cavalcanti et al., 2007). Protein content of the crude extracts was determined using the Bradford (1976) protein assay, with bovine serum albumin (BSA) as a standard.

Chitinase activity CHI (PR-3; EC 3.2.1.14) was determined by adding 70 µL of suitably diluted crude extract with 130 µL of 50 mM sodium acetate pH 5.2 and 60 µL of CM-Chitin-RBV (2 mg mL^{-1}), a polymeric carboxymethyl-substituted chitin, labelled covalently with Remazol Brilliant Violet 5R (CM–Chitin–RBV, Loewe, Biochemica, Germany) used as substrate, in microplates of 96 wells with a capacity of 350 µL. After incubation at 35^{o} C for 80 min, samples were acidified with 50 µL of 0.5 N HCl, cooled in ice bath for 10 min and centrifuged (1,450 g for 10 min). Absorbance of the supernatant at 492nm was recorded and the results were expressed as UA. One unit of CHI activity was defined as the variation of one absorbance unit at 492 nm per milligram of soluble protein per minute (UA mgP^{-1} min^{-1}). Assays were carried out in triplicate.

The activity of beta-1,3-glucanase GLU (PR-2; EC 3.2.1.39) was measured using similar method, with the exchange of the substrate for CM-Curdlan-RBB (4mg mL^{-1}) and the adjustment of the rate of enzyme extract to 100µL (minus the volume of acetate buffer in order to adjust the final volume to 310µL per cavity). To promote the hydrolytic action of beta-1 3-glucanase was adopted incubation period of 35° C for 100 min. Absorbance of the supernatant at 620 nm was recorded and the results were expressed as UA. One unit of CHI activity was defined as the variation of one absorbance unit at 620

nm per milligram of soluble protein per minute (UA mgP^{-1} min^{-1}). Assays were carried out in triplicate.

The activity of PPO (EC 1.10.3.2) was determined by adding 50 µL of the crude extract to 3 mL of a solution containing 100mM potassium phosphate buffer, pH 6.5 and 25mM pyrocatechol. The increase of absorbance at 410 nm, for 10 min at 30° C, was measured (Gauillard et al., 1993). One PPO unit was expressed as the variation of absorbance at 410 nm per milligram of soluble protein per minute (UA mgP^{-1} min^{-1}).

For lignin quantification, the assay described by Monties (1989) was used with minor modifications (Cavalcanti et al., 2006). Samples of 0.2 mg dry leaf tissue were powdered with liquid nitrogen and incubated with 85% acetone for 48 h. After centrifugation at 7500 g for 15 min at 4°C, the green supernatant was discarded and the remaining ketonic precipitate was air-dried, re-suspended in 5 mL thioglycolic acid (TGA) (SIGMA) prepared in 2N HCl (1:10, v/v) and left for 4 h at 25°C. Next it was centrifuged at 7500 g for 15 min at 4°C and the resulting supernatant transferred to a fresh 20 mL tube to which 200 mL 10M HCl was added. After incubation in an ice-bath for 4 h, this mix was centrifuged (7500g, 30 min, 7°C), the pellet was homogenized in 5 mL 0.5N NaOH, and the absorbance at 280nm was measured. TGA derivatives (acid-soluble lignin) formed were quantified by comparison with a standard curve prepared with known amounts (10–100 mg mL^{-1}) of 2-hydroxypropyl ether. Assays were done in triplicate and data expressed as micrograms per milligrams of dry weight.

2.6 Experimental design and statistical methods

The disease severity was measured in greenhouse conditions, experiments (twice) were arranged in randomized block designs with four blocks, and one experimental unit (plot) consisted of four 3 L pot containing

two plants. For the biochemical determinations, experiments were arranged in randomized block designs with three blocks, in a factorial scheme and one experimental unit (plot) consisted of four 3 L pot containing three plants each.

For analysis by reverse transcription–polymerase chain reaction (RT–PCR) experiments plants were arranged in randomized blocks designs with three blocks, and one experimental unit (plot) consisted of four 1.5 L pot containing two plants each.

Variance analysis was run using SAS (Statistical Analysis Systems Inc., Cary, NC, USA) statistical software. Means were separated using Tukey's test at P value less than 0.05 using Sisvar, a statistical tool purchased from the Federal University of Lavras, Minas Gerais, Brazil.

3 RESULTS

3.1 Effects of foliar sprays of NEFID and ASM on the disease severity of bacterial spot and plant development under greenhouse conditions.

First symptoms of bacterial spot of tomato inoculated with *X. vesicatoria* were detected 72 hours after inoculation. A coffee leaf formulation has shown to control tomato bacterial spot and this response occurred as early as 7 days after inoculation with 35% disease severity reduction, a significatively reduction (61%) was obtained by the commercial plant defense activator Actiguard (Acibenzolar-S-methyl) (Figure 1).

The plant growth was measured at 3, 7, 10, 13, 16 and 20 days after spraying. Plants treated with the disease resistance elicitors had previously shown to have a larger canopy than water pre-treated controls. By measuring plant height over time and analyzing the overall growth both elicitors, coffee leaf formulation – NEFID and acibenzolar-S-methyl, improved plant growth (9.68 and 13.56%, respectively) at 13 DAS (Table 1), after this time, only plants sprayed with ASM had shown enhanced in the seedlings growth compared with water pre-treated plants. The commercial elicitor also improved leaf area (93%) while NEFID did not affect leaf growth (Figure 2). The leaf area was measured at the end of the experiment, 20 days after spraying.

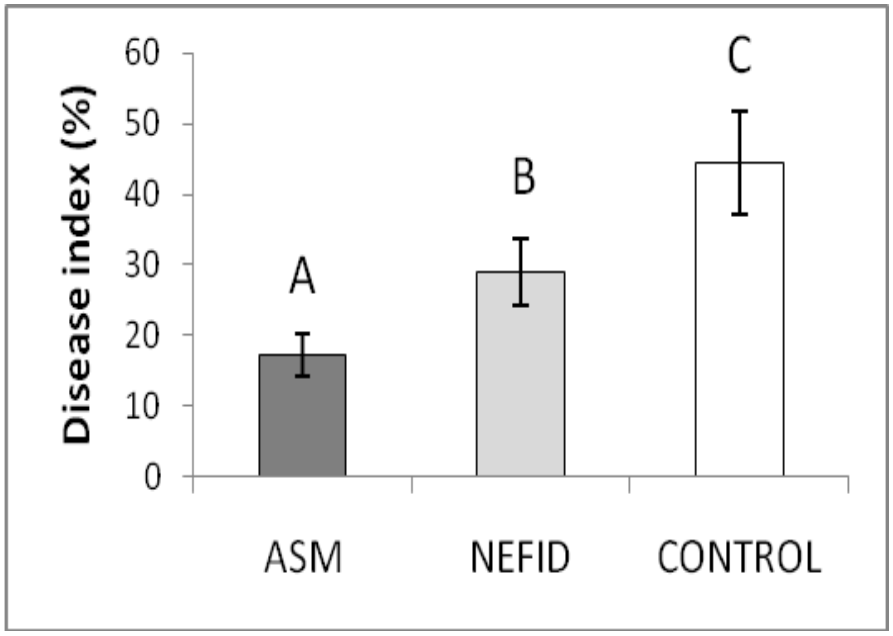

FIGURE 1 Coffee leaves formulation (NEFID) and the commercial disease resistance inducer ASM (Acibenzolar-S-methyl) reduced the severity of tomato bacterial spot caused by *X. vesicatoria* in a susceptible cultivar, 'St. Cruz Kada' compared to the water pre-treated seedlings (control) seven days after inoculation. Tested substances were sprayed on leaves 25 days after planting and plants were inoculated 3 days later. Bars followed by the same letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$).

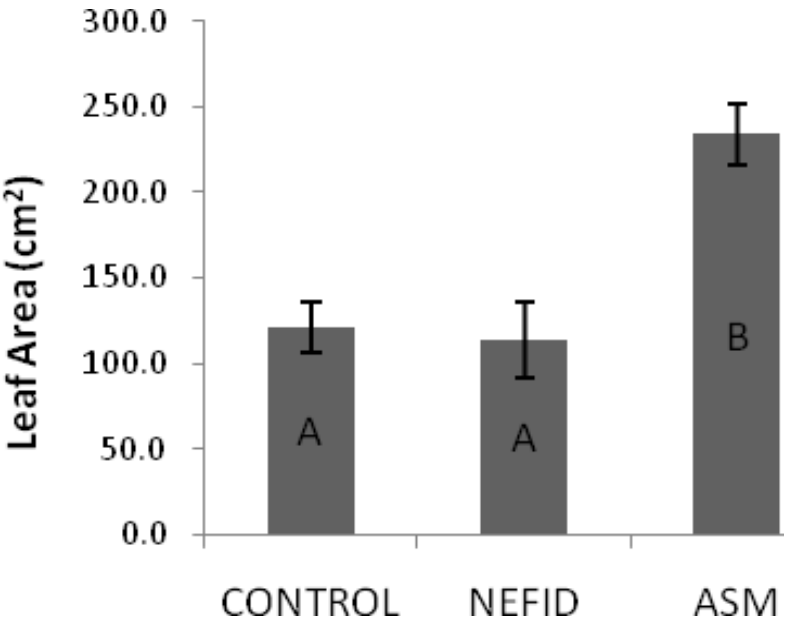

FIGURE 2 Effect of coffee leaves formulation (NEFID) and acibenzolar-S-methyl (ASM) on tomato leaf area. Bars followed by the same letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$).

TABLE 1 Effect of coffee leaves formulation (NEFID) and acibenzolar-S-methyl (ASM) on tomato seedlings height.

Treatment	Days after spraying				
	3	10	13	16	20
	----------(cm)----------				
NEFID	26.70 a	52.83 a	58.62 b	64.42 a	78.33 a
ASM	25.83 a	50.04 a	56.86 b	70.25 b	83.62 b
CONTROL	27.12 a	50.58 a	51.62 a	60.62 a	73.25 a
Mean	26.55	51.15	55.70	65.09	78.40
Ftest $_{\textbf{treatment}}$	1.32^{ns}	0.95^{ns}	6.03**	9.93**	17.57**
DMS	2.001	5.296	5.172	5.365	6.490
CV (%)	7.49	10.28	9.22	8.19	8.59

The values were the means of four replicates. Means in the same column followed by the same letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$); HSD = honestly significant diference; ^{ns}nonsignificant at ($P>0.05$); CV = coefficient of variation **difference at ($P \leq 0.01$).

3.2 Regulation of PR-1 and beta-1,3-glucanase gene expression by NEFID and ASM.

In order to investigate the similarities in the resistance induction between both tested products, the expression level of key genes (PR-1, β-1,3-glucanase) were investigated over a time course (12, 24, 48, 72, 96, 144 hours). Tomato β-1,3-glucanase gene expression was clearly induced at the earlier stages (12 hours) after spraying the tomato seedlings with NEFID or ASM (3.2 and 1.6 fold changes, respectively) in comparison with the water-sprayed control (Figure 3a). However, treatment with NEFID persisted higher than that of control plantlets at 24, 48, 72 and 144 hours after spraying with 2.02, 2.08,

1.71 and 2.04 fold changes, respectively, while treatment with ASM showed increase on GLU gene expression at 72 and 144 HAS (1.59 and 2.52 fold changes) and no differences compared with control at 24, 48 and 96 HAS.

PR-1 gene expression from NEFID and ASM treated plants initially, at 12 HAS, had demonstrated to be up-regulated with 2.04 and 2.08 fold changes, respectively (Figure 3b). At 24 and 48 HAS there were no deferential regulation compared with the water pre-treated plants ($P \leq 0.05$). At 72 and 144 HAS there were a slight difference at PR-1 expression level between NEFID (1.27 and 1.19) or ASM (1.27 and 1.20 fold changes, respectively) for treated and water pre-treated plants.

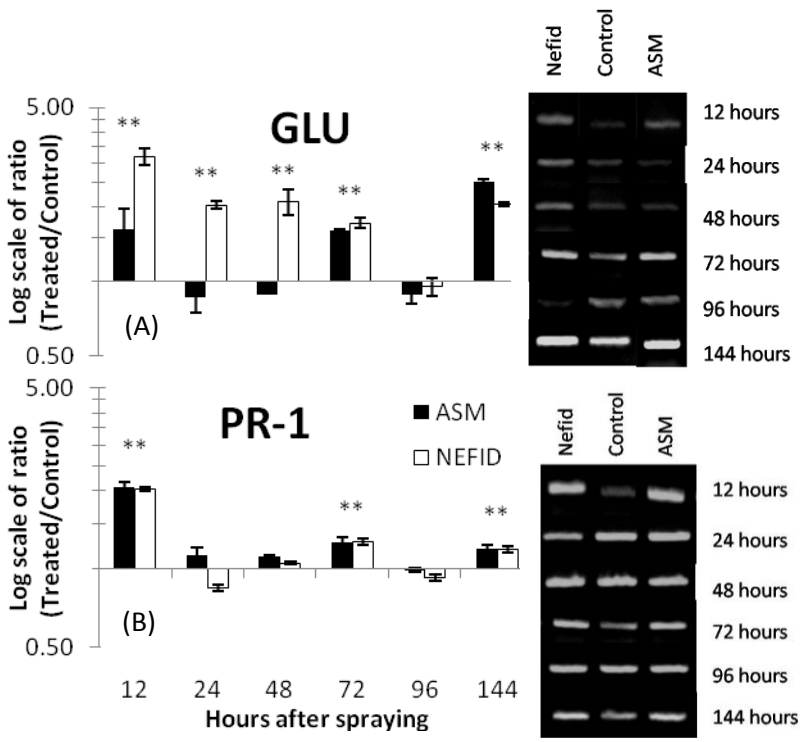

FIGURE 3 ASM and NEFID upregulate GLU and PR-1 levels in tomato. Tomato β-1,3-glucanase gene is upregulated in seedlings sprayed with ASM at 12, 72 and 144 hours after spraying and 12, 24, 48, 72 and 144 hours in plants sprayed with NEFID (a). While PR-1 gene is upregulated at 12, 72 and 144 HAS in both plants sprayed with ASM or NEFID (b). Gene expression ratios are quantified from RT-PCR results. Black bars represent ASM; white bars represent NEFID treatment. **P ≤ 0.01, treated versus controls (n = 4, mean ±SD).

3.3 Effects of foliar sprays of NEFID and ASM on the activities of GLU, CHI, PPO and lignin deposition on tomato plants.

Whereas the up-regulation of typical SAR gene (PR-1) was also associated to the expression of chitinase and β-1,3-glucanase genes, post-transcriptional regulation or modulation may affect the overall pathogenesis related protein expression, therefore those enzymes were measured in a 0-240h time-course (Fig. 4). Those studies corroborate gene expression profile, with early protein expression (24-48HAS).

β-1,3-glucanase (GLU) activity at the earliest time point (24 hours) experienced an abrupt increase in values around five and three time higher than the GLU activity of control, for plants pre-treated with ASM and NEFID, respectively (Figure 4a). Treatment of tomato plants with ASM triggered an expression of GLU in all sampled time points which was a similar pattern found for NEFID treatment (induction from 0-120HAS).

Both NEFID and ASM treatments were able to maintain a higher level of chitinase activity at 24HAS. After this early pick, the chitinase activity of tomato plants pre-treated with NEFID or ASM did not differ remarkably from those of plants pre-treated with water (control) for all of after time points, suggesting the tested products did not induce the effect of chitinase for a long time period.

While pathogenesis related enzymes represent an important arsenal for the pathogen control, other pathogen toxic compound synthesis is also triggered by abiotic elicitors such as phenolics and a key enzyme is the polyphenoloxidase (PPO) which was expressed at 24-48HAS for both ASM and NEFID. ASM also induced a later response of this enzyme (240HAS).

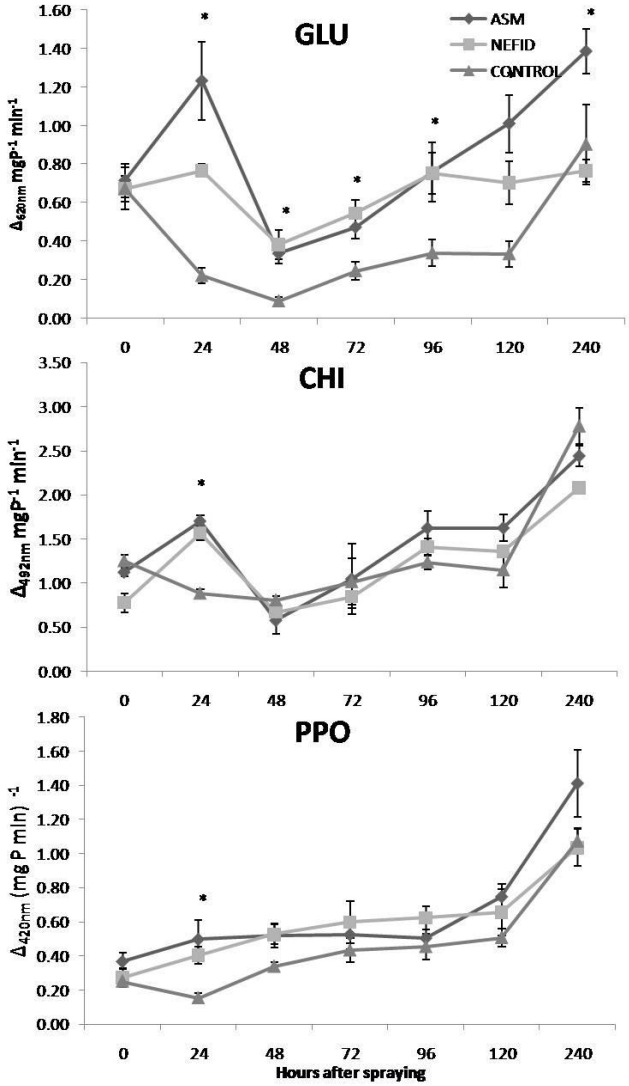

FIGURE 4 Activity of β-1,3-glucanase (GLU) (A), chitinase (CHI) (B) and polyphenol oxidase (PPO) (C) was induced at the earlier stages (24 hours) after spraying the tomato seedlings with NEFID or ASM in comparison with the water-sprayed control. Enzymatic responses were evaluated 0, 24, 48, 72, 96, 120, 240 hours after spraying. Error bars indicate standard deviations.

Although the GLU, CHI and PPO activity of tomato plants pre-treated with ASM, NEFID or water (control), showed significantly ($p \leq 0.05$) different patterns within the tested treatments, the interaction between treatment and pathogen inoculation (*Xanthomonas vesicatoria*) did not showed a significant difference, suggesting the bacterium itself in this plant pathogen interaction did not influence the effect of the treatment. In turn, the inoculation with *X. vesicatoria* increases almost two times the GLU activity, at seven days after the inoculation, in tomatoes plants (data not shown).

Lignin content for ASM and NEFID sprayed plants showed a significant ($p \leq 0.05$) increase in lignin content compared to water-treated controlsThe ASM-treated plants showed a significant increase of lignin deposition between 17 and 23 days after spraying in values around two times higher than those measured at the 10-17 DAS interval and higher than the control that remained nearly constant (Figure 5).

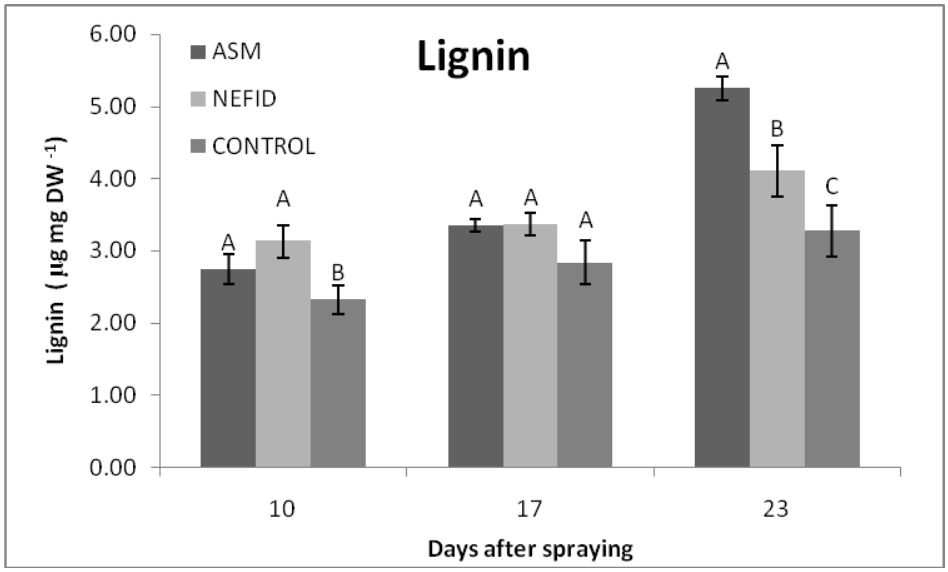

FIGURE 5 ASM and NEFID induced a higher lignin deposition in tomato plants cv. Santa Cruz 'Kada' in comparison with the water-sprayed control. Lignin depositions were evaluated 10, 17 and 23 days after spraying. Error bars indicate standard deviations.

4 DISCUSSION

The worldwide damaging impact of bacterial spot (*Xanthomonas vesicatoria*) on tomato production has resulted in different stradigies for plant protection based on fungicides (e.g. mancozeb), biocontrol agents (e.g. *Bacillus sp*) and induced resistance elicitor (e.g. ASM) to be used individually or in combination (Roberts et al., 2008). Induction of systemic acquired resistance (SAR) is generally considered as part of an integrated pest management program because of its broad spectrum and multi-site disease control activity (Da Rocha & Hammerschmidt, 2005). A coffee based leaf formulation (NEFID) exhibits disease reduction properties similar to the commercial product ASM (Figure 1) and comparable to previous findings (Cavalcanti et al., 2006). While ASM and other disease resistance inducers can efficiently control diseases redirecting primary metabolism lowering of biomass accumulation and harvest yields are not always observed (Bostock, 2005). With respect to plant height and leaf surface area over a five point time-course up to 20 days after spraying growth retardation was not observed compared to the untreated control at any time. Furthermore, both ASM and NEFID improved plant height from 13 to 20 das and at 16 das, respectively and ASM improved leaf area.

The mechanisms by which plants detect pathogen presence and initiate a defense response are poorly understood and are the subject of intensive investigation (Anderson et al., 1998). Resistance mechanisms are activated in uninfected tissues (systemic acquired resistance, SAR) which provide the plant more resistant to secondary infection by the same or unrelated pathogens (Ryals et al., 1996). SAR is associated with the increased expression of a wide array of defense genes, among which are those encoding various classes of pathogenesis-related (PR) proteins (Cutt et al., 1992; Stintzi et al., 1993). The functions of some of these proteins during the resistance response

are known, such as the antimicrobial activity of the β-l,3-glucanases (PR-2) and chitinases (PR-3). In contrast, the functions of other PR proteins are less well understood, although they are thought to be integral components of disease resistance. Due to the strict correlation between *PR* gene induction and the development of disease resistance, the *PR* genes are commonly-used molecular markers for the defense response (Anderson et al., 1998).

As expected from previous experiments (Mandal et al., 2008), typical disease resistance responses were present and the SAR marker protein gene PR-1 was up-regulated as early as 12 hours after treatment with ASM and NEFED. Although PR-1's function has not been clearly defined, the up regulation of a SAR marker gene is suggestive of other responses belonging to the same defense pathway being operative. Both chitinase and glucanase are common SAR associated responses and their gene and protein expression levels were investigated. Both were found to be operative for both tested treatments, however in different time-courses. For glucanase, the expression at early time points (up to 96 h) in both treatments (Figure 4a) corroborates with the timeframe necessary from treatment of the elicitors to the inoculation with the pathogen to achieve disease control and was also over-expressed in the induction of resistance against bacterial spot in tomato after treatment with ASM (Cavalcanti et al., 2006). Thus, once the pathogen infects the plant, the defense machinery is ready to hinder the disease onset. The enzyme also has a lysozyme activity acting to hydrolyze bacterial cell walls (Mauch et al., 1988).

In our experiment chitinase was found to be over-expressed in a pattern similar to glucanase (Figure 4a and 4b) even though plants were not challenged with a chitin containing fungal pathogen. The potential of using both ASM and NEFID each one to control fungal diseases has already been reported (Santos et al., 2007) and although NEFID has not been tested against

tomato fungal pathogens, the coffee leaf formulation is expected to be effective in an integrated pest management program against other tomato pathogens.

The use of resistance inducers such as ASM and NEFID activates not only typical SAR responses but also the phenylpropanoid pathway and intermediate compounds such as poliphenols that have a deleterious effect on plant pathogens. It was found to be an early response (24 and 48 h after treatment) but is likely to have a pivotal role in the resistance to bacterial infection. The down-regulation of a gene coding for polyphenol oxidase was correlated with higher *Pseudomonas syringae* infection in tomato (Thipyapong et al., 2005).

One of the products of the phenylpropanoid pathway is the polyphenolic compound lignin which is associated with resistance to bacterial spot in tomato after treatment with ASM (Cavalcanti et al., 2006). This polymer accumulates in the tomato cell wall after treatment with pathogen derived extracts or chitosan delaying the onset of infection and is associated to an early induction of polyphenoloxidase (Mandal & Mitra, 2007).

Both the disease control levels and biochemical responses induced by NEFID and ASM followed a similar pattern with induction of salicylic acid and phenylpropanoid pathways with responses previously reported as being operative against bacterial infection, albeit no adverse effects on the plant development was detected. The observed induction of plant responses associated with growth and defense reported here indicate that the the coffee leaf formulation could serve as a biological alternative for tomato growers in the management of bacterial spot with the distinct advantage over ASM in that it can be utilized in organic farming (Santos et al., 2007).

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CHAPTER 3

Defense gene expression induced by the biocontrol coffee-leaf formulation in tomato

ABSTRACT

Plant formulations have the potential to activate defense related genes and the mechanism of action can be monitored in plants by using microarray technology. A coffee leaf formulation has shown its potential in the control of both bacterial and fungal pathogens in different hosts. The transcriptional response of coffee leaf eliciator (NEFID) in plants was evaluated by using a tomato gene chip. A total of 268 genes were found to be differentially regulated with a majority up-regulated and encoding signal transduction, defense responses, and transcription factors. Since salicylic- and jasmonic-acid signaling components were not differentially transcribed with elicitor treatment while mitogen activated proteins (MAP3K and MAPKK) as well as calcium dependent (calmodulin and phosphatidylinositol) signaling components were up regulated, a SA/JA-independent transduction sequence for PR accumulation is predicted. Chitinases, glucanases and peroxidases (PR-4), with reported activity against pathogens, were transcriptionally up-regulated and corresponding enzyme activities were over-expressed as early as 24 h post treatment and remained elevated for up to five days subsequently. These results demonstrate the ability of the coffee leaf formulation (NEFID) to differentially regulate gene expression in tomato.

Keywords: *Solanum lycopersicum*, systemic acquired resistance, natural formulation, microarray approach

RESUMO

Formulações de plantas tem o potencial de ativar genes relacionados a defesa e o seu mecanismo de ação pode ser monitorado nas plantas utilizando-se a tecnologia dos microarranjos. Uma formulação a base de folhas de café tem demonstrado potencial de controle de patógenos bacterianos e fúngicos em diferentes hospedeiros. A resposta transcricional em plantas de tomateiro mediada por uma formulação baseada em folhas de café (NEFID) foi avaliada utilizando-se um chip de microarranjos de tomate. Um total de 268 genes foram diferencialmente regulados, em sua maioria super-expressos, codificando principalmente para a transdução do sinal, resposta de defesa e fatores de transcrição. Uma vez que componentes sinalizadores da rota do ácido salicílico e jasmônico não foram diferencialmente transcritos com o tratamento pelo eliciador (NEFID) enquanto proteínas ativadas por mitógenos (MAP3K e MAPKK) assim como componente sinalizadores dependentes de cálcio (calmodulina e fosfatidilinositol) foram super-expressos, uma sequência de trasndução, independente de SA/JA, para a acumulação de PR proteínas é predita. Quitinases, glucanases e peroxidases (PR-4), os quais tem atividade antipatogênica relatada, foram transcricionalmente super-expressas e atividades enzimáticas correspondentes as estas enzimas foram também super-expressas 24 horas após a pulverização do eliciador e manteve-se elevada por até cinco dias subseqüentes. Estes resultados demonstram a habilidade da formulação à base de folhas de café em regular a expressão gênica em tomateiro.

Palvras-chave: *Solanum lycopersicum*, resistência sistêmica, formulação natural, técnica de microarranjos

1 INTRODUCTION

Tomato (*Solanum lycopersicum* L., previously *Lycopersicon esculentum* Miller) is among the economically most important crops worldwide (Souza & Resende, 2003). Furthermore the plant species is diploid, has a short generation time, a feasible transformation technology, rich available genomic information and therefore represents a model plant for molecular biology studies (Barone et al., 2008). The crop is affected by over 200 pathogens (Jones et al., 1991) and researchers have directed efforts towards tomato disease control, especially bacterial spot, caused by *Xanthomonas euvericatoria* (Wang et al., 2004).

The disease control relies on the use of agrochemicals and plant resistance. Although the main agrochemicals streptomycin sulfate and copper-based products have proven to be efficient, due to its overuse, resistant populations have arisen (Gore & Garro, 1999). From a recent breeding program, a tomato cultivar has already achieved resistance to the different pathogen races (Souza et al., 2008) but the breeding line is not yet available to growers and it is not impossible that a new race not considered in the breeding would lead to failure in the disease control from the exclusive use of bacterial spot resistant plants.

Alternatively, disease control may be achieved by the eliciation of plant resistance genes prior to pathogen attack. Several products have shown elicitation activity such as the salicylic acid analogue acibenzolar-S-methyl (ASM). When tomato plants were sprayed with ASM in the field where bacterial spot occurred, an increase of up to 134% in fruit yield was observed and the results were comparable to the standard bactericide (Louws et al., 2001).

Other elicitors may be based on inactivated pathogen (Cavalcanti et al., 2007) or even a combination of both inactivated pathogen and plant oligomers, and a product based on rust infected coffee leaf formulation, so-called NEFID, has recently been patented (I.N.P.I., Protocol number 0000220604167501, "Formulation for resistance induction"... Aug/2006) that contains both elicitors. NEFID was effective in the control of coffee rust and phoma spot, bacterial blight in cotton as well as bacterial spot in tomato. The underlying mechanism was the accumulation of pathogenesis-related proteins, phytoalexin production and lignin content in the leaves following plant sprays (Barguil et al., 2005, Ishida et al., 2007; Santos et al., 2007).

An important strategy to probe not only the defense-related events but all changes in the plant metabolism due to an elicitor treatment is the use of microarrays (Moore et al., 2005). When challenged with a yeast elicitor, tomato was found to induce SAR-dependent PRs (chitinase and glucanase), an up-regulation of cytochrome P450 and a down-regulation of cell wall loosening and expansion (expansin, xyloglucan endoglycosl transferase, polygalacturonase) (Jiang et al., 2009). SAR-elicited plants were yet involved in acclimatory responses to stress, such as recovery of the cell redox balance (glutathione transferase, UDP glycosyl transferase and glutaredoxins), intracellular stress signaling, improvement of pathogen recognition, and promotion of metabolic changes (Blanco et al., 2008). On the other hand, plants treated with fusicoccin displayed an elicitation of plant defense genes as well as the expression of a jasmonic acid biosynthesis gene which was not related to the defense signaling but associated to the inhibition of the biosynthesis of photosynthetic pigments and photosynthetic activity (Frick & Schaller, 2002).

The plant formulation NEFID when sprayed on tomato plants protected them against bacterial spot and we have characterized the overall transcriptomics change after treatment using the *Solanum lycopersicon*

oligomer microarray chip TOM2. In this study we found a changed regulation of 268 genes and report that bacterial treatment induces a SA or JA independent activation of PR genes (chitinase, glucanase and peroxidase) as well as an over-expression of the corresponding proteins as early as 24h after treatment. We also addressed the other genes with changed regulation such as cell wall modification, transcription factor and stress alleviation genes and the the possible impacts on phenotype.

2 MATERIAL AND METHODS

2.1 Plant material and NEFID formulation

Tomato seeds (*Solanum lycopersicum* var. Santa Cruz Kada), purchased from Isla Sementes Ltda (Porto Alegre — Rio Grande do Sul, Brazil) were surface sterilized in 1% (v/v) ethanol for 3 min, followed by a 1.0 g L^{-1} sodium hypochlorite solution for 1 min and then rinsed thorough with distilled water. Seeds were sown in 1.5 L pots containing 400 g of the potting mix Sunshine® All-Purpose Planting Mix (Sun Gro Horticulture, Vancouver, CA), fertilized with 5 g of Osmocote fertilizer (Scotts-Sierra Horticulture, Marysville, OH, USA), irrigated to field capacity daily. Plants were grown under a controlled temperature (25º C ± 4), relative humidity 40 ± 10% and light (200 μmol m^{-2} s^{-1}) by using a combination of metal halide and high sodium pressure lamps set for 14 h/day light period. For enzyme assays and disease severity measurements, plants were greenhouse grown in Plantmax® soil (Eucatex, Paulinia, SP, Brazil) with a mean temperature of 28 ± 3º C day/23 ± 3º C night, relative humidity of 40 ± 3%/85 ± 3%, and a 12 h photoperiod, at 450–500 mmol m^{-2} s^{-1} maximum photon flux densities, measured at plant level (IRGA, model LCA-4, Hoddesdon, UK).

The coffee formulation (NEFID) contains field-collected coffee leaves (*Coffea arabica*), from soil surface (due to disease, harvest fruit and/or other stresses) and selected for powder production. A hundred grams of this powder is mixed with 1000 mL distilled water, boiled in reflux and filtered through a sieve of 400 meshes. The filtrate of leaves is sampled and stored at -20ºC. The formulation based on NEFID is patented by Resende et al. (2006) **(I.N.P.I., Protocolo número 0000220604167501, FORMULAÇÃO PARA INDUÇÃO DE RESISTÊNCIA... 02 de Agosto de 2006).** Twenty-five days

after planting, (plant height ca. 20±4 cm), tomato leaves were sprayed with either NEFID or H_2O (control).

2.2 Plant sampling and RNA extraction

Plant tissue was harvested and frozen in liquid nitrogen and stored at -80ºC for RNA extraction.

Samples were ground in mortar and pestle under liquid nitrogen and RNA extracted following the protocol of RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) including the RNase-free DNAse treatment step from the same manufacturer. The clean RNA was quantified and stored at -80ºC until use.

2.3 Microarray analysis

Microarray hybridizations consisted of four biological replicates, one of which consisted of a dye swapping. The previously obtained target RNA was transcribed to aRNA in a three step transcription using Amino-Allyl aRNA Amplification Kit (Ambion, Austin, TX, USA) and labeled with NHS dyes Cy3 or Cy5 (Amersham Biosciences, Little Chalfont Buckinghamshire, UK) according to the manufacturer's protocol. Samples were assayed on the tomato TOM2 oligo-arrays printed at University of Arizona. Each chip containing 12,160 70-mer oligonucleotide elements (http://ted.bti.cornell.edu/cgi-bin/TFGD/array).

Slide pre-hybridization was performed according to the manufacturer, whereas hybridization and post-hybridization followed the *Arabidopsis thaliana* protocol (Zhang et al., 2007). The arrays were scanned using a GenePix 4100 array scanner (Axon Instruments, Sunnyvale, CA, USA). Spot statistical analysis was performed according to the manufacturer's guidelines (Gene-Spring 7.0; Silicon Genetics, Redwood, CA, USA). A 40% change, either up- or down-regulation, in the expression level compared with the control

was selected as the threshold for a gene to be classified as altered in response to NEFID treatment. Only genes that passed the Flag Filtering, identified as present (Gene-Spring 7.0), and passed the T-test P-values 0.10 was considered differentially regulated with the NEFID treatment.

2.4 Validation of microarray result

For the microarray result validation, first strand cDNA was synthesized from 5μg of total RNA following Zhang et al. (2007) and PCR performed using the (5'-3') primers designed based on genes with significatively changed expression (Table 1). Seven genes were chosen (Table 1) and the primers designed based on the UNIGENE used to generate each microarray probe (Tomato functional genomics database, available at http://ted.bti.cornell.edu/).

TABLE 1 Primers used for RT-PCR

Gene	Name	Forward	Reverse
Endo-1,3-beta-glucanase	SGN-U212943	ACAGCTCATACATGGCCTTCT	ATTGGGCTTCTTGGTTGTGGTTGG
Pectinesterase	SGN-U214672	CGCATGGGCTGATTGCATTGAACT	CGACGACCGACGATGCAACAATTT
Chitinase	SGN-U224778	ATGGCGGAAACTGTCCTAGTGGAA	ACATGGTCTACCATCAGCTTGCCA
Calmodulin	SGN-U212854	TGCTGGTAGTTGTGGGAGTTGAGA	AGCTCCTTAGTCGTGATGCAACCT
Peroxidase	SGN-U213351	ACGGAGCAAGCGACAATTGACAAC	CGATTGATTCACCGCAAAGCTCGT
Proteinase inhibitor	SGN-U213363	CGGAGAATCTGAATGGGTAAGCGA	ACAAGCCGTGGTAAAGGTCCACAA
Glutatione S-transferase	SGN-U216884	TGTCCCAACCTTCTCGTGCAGTTA	TGAGTGATGCCAGTCCAACACAGA
Actin	SGN-U226051	TTGACTGAGGCACCACTTAACCCT	GCTTTCAGGTGGTGCAACGACTTT

Agarose gel electrophoresis images were taken by Kodak Gel Logic 100 Imaging System (Fisher ScientiWc, Houston, TX, USA) and the band intensity quantified by Image J 1.33u (http://rsb.info.nih.gov/ij/, National Institute of Health, USA).

2.5 Enzyme extraction, PR-protein, defense enzyme assay

Tomato fresh leaf (1.0 g), treated with the natural formulation (NEFID) and control (water sprayed), collected at 0, 24, 48, 72, 96 and 120 hours after spraying (HAS), was homogenized for 5 min in a mortar with a pestle in 3 mL of ice-cold 50mM sodium acetate buffer pH 5.2, containing 0.1mM EDTA. After filtration, the homogenate was centrifuged at 13,000g for 15 min and the supernatant (crude extract) used as the source of enzymes. All the steps were carried out at 0–4^{o} C (Cavalcanti et al., 2007). Protein content of the crude extracts was determined using the Bradford (1976) protein assay, with bovine serum albumin (BSA) as a standard.

The activity of guaiacol peroxidase POX (EC 1.11.1.7) was determined by adding 25 mL of the crude-extract preparation to 2mL of a solution containing 50mM sodium acetate buffer, pH 5.2, 20mM guaiacol and 20mM hydrogen peroxide. After incubation at 30^{o} C for 10 min, the absorbance was read at 480nm (Urbanek et al., 1991). One POX unit of activity (UA) was expressed as the variation of one unit of absorbance at 480nm per milligram of soluble protein per minute (UA mgP^{-1} min^{-1}).

Chitinase activity CHI (PR-3; EC 3.2.1.14) was determined by adding 70 µL of suitably diluted crude extract with 130 µL of 50 mM sodium acetate pH 5.2 and 60 µL of CM-Chitin-RBV (2 mg mL^{-1}), a polymeric carboxymethyl-substituted chitin, labelled covalently with Remazol Brilliant Violet 5R (CM–Chitin–RBV, Loewe, Biochemica, Germany) used as substrate, in microplates of 96 wells with a capacity of 350 µL. After incubation at 35^{o} C

for 80 min, samples were acidified with 50 μL of 0.5 N HCl, cooled in ice bath for 10 min and centrifuged (1,450 g for 10 min). Absorbance of the supernatant at 492nm was recorded and the results were expressed as UA. One unit of CHI activity was defined as the variation of one absorbance unit at 492 nm per milligram of soluble protein per minute (UA mgP^{-1} min^{-1}). Assays were carried out in triplicate.

The activity of beta-1,3-glucanase GLU (PR-2; EC 3.2.1.39) was measured using similar method, with the exchange of the substrate for CM-Curdlan-RBB (4mg mL^{-1}) and the adjustment of the rate of enzyme extract to 100μL (minus the volume of acetate buffer in order to adjust the final volume to 310μL per cavity). To promote the hydrolytic action of beta-1 3-glucanase was adopted incubation period of 35° C for 100 min. Absorbance of the supernatant at 620 nm was recorded and the results were expressed as UA. One unit of CHI activity was defined as the variation of one absorbance unit at 620 nm per milligram of soluble protein per minute (UA mgP^{-1} min^{-1}). Assays were carried out in triplicate.

2.6 Experimental design and statistical methods

For the biochemical determinations, experiments were arranged in randomized block designs, with three blocks, one experimental unit (plot) consisted of four 3 L pot containing three plants each. For analysis by microarray and reverse transcription–polymerase chain reaction (RT–PCR) experiments, plants were arranged in randomized blocks designs, with three blocks, and one experimental unit (plot) consisted of four 1.5 L pot containing two plants each. Variance analysis was run using SAS (Statistical Analysis Systems Inc., Cary, NC, USA) statistical software. Means were separated using Tukey's test at P value less than 0.05 using Sisvar, a statistical tool purchased from the Federal University of Lavras, Minas Gerais, Brazil.

3 RESULTS

3.1 Regulation of gene expression by NEFID

In order to provide insight into underlying mechanisms responsible for the induction of resistance by a natural formulation based in *Coffea arabica* leaves, genome-wide analysis of gene expression was performed using oligonucleotide microarray slides. Three independent microarray analyses were executed. Labeled mRNA from tomato leaves tissue harvested at 12 hours after spraying with the natural formulation of coffee leaves (NEFID) or water was hybridized with microarray slides, with 12,000 spots, designed for over 11,000 tomato unigenes. Changes in RNA levels in response to NEFID treatment (i.e. induced or repressed) were assessed using oligo microarray slides (TOM2).

A total of 268 genes were differentially expressed with NEFID treatment compared to the water control, most of which (80%) were up-regulated. The number of genes with increased signals was 215 and that with decreased signals was 53. Microarray responses were validated by RT-PCR analysis of selected genes. All seven genes tested showed a similar fold change (Figure 1).

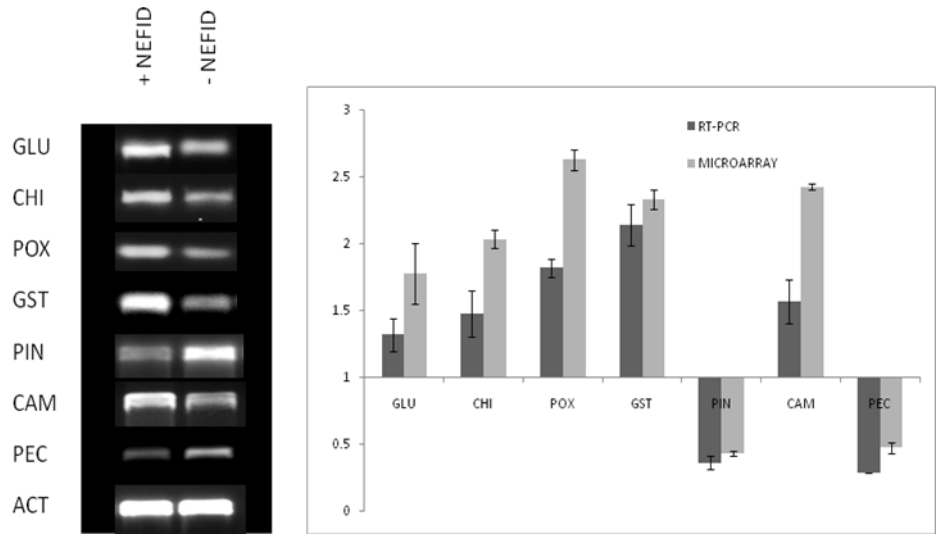


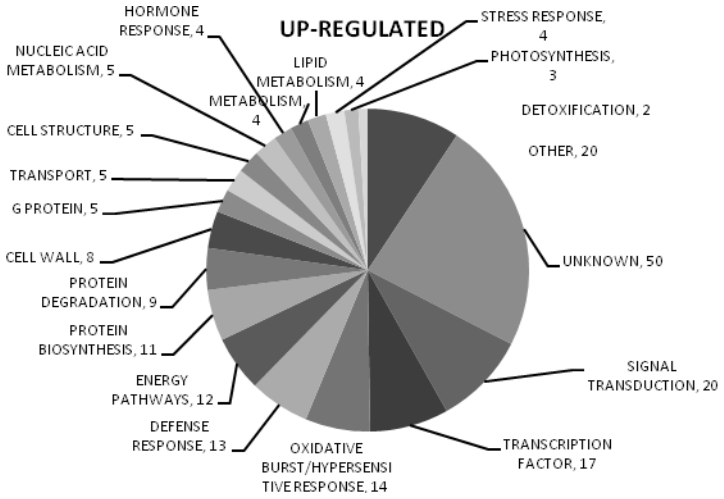
FIGURE 1 Expression level of genes of significatively changed regulation (P<0.10 and ratio>1.4 or <0.6) from the microarray data compared to the RT-PCR: endo 1,3 beta-glucanase SGN-U212943 (GLU), Chitinase SGN-U224778 (CHI), Peroxidase SGN-U213351 (POX), Glutatione S-transferase SGN-U216884 (GST), Proteinase inhibitor SGN-U213363 (PIN), Calmodulin SGN-U212854 (CAM), Pectinesterase SGN-U214672 (PEC) e Actin (ACT).

3.2 Putative identification and functional category assignment

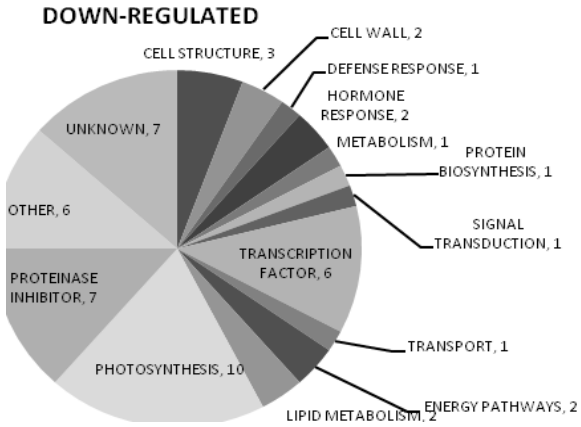
The putatively known genes for which significant expression changes were observed and tentatively arranged in categories according to its reported function. The putative function of the NEFID-regulated genes fits into the following categories, "signal transduction" (9% of up-regulated genes, 2% of down-regulated genes), "transcription factor" (8% of up-regulated genes, 11% of down-regulated genes), "oxidative burst/hypersensitive response" (7% of up-regulated genes), "defense response" (6% of up-regulated genes, 4% of down-regulated genes), "energy pathways" (6% of up-regulated genes, 4% of down-regulated genes), "protein biosynthesis (5% of up-regulated genes, 2% of

down-regulated genes), “protein degradation” (4% of up-regulated genes), “cell wall” (4% of up-regulated genes, 4% of down-regulated genes), “cell structure” (2% of up-regulated genes, 5% of down-regulated genes), “metabolism” (2% of up-regulated genes, 2% of down-regulated genes), “hormone response” (2% of up-regulated genes, 4% of down-regulated genes), “transport” (2% of up-regulated genes, 2% of down-regulated genes), “lipid metabolism” (2% of up-regulated genes, 4% of down-regulated genes), “photosynthesis” (1% of up-regulated genes, 19% of down-regulated genes), “nucleic acid metabolism” (2% of up-regulated genes), “G protein” (2% of up-regulated genes), “stress response” (2% of up-regulated genes), “detoxification” (1% of up-regulated genes), “proteinase inhibitor” (13% of down-regulated genes), “others” (9% of up-regulated genes, 11% of down-regulated genes), “unknown” (23% of up-regulated genes, 13% of down-regulated genes) as shown in Figure 2.

The five categories with the larger number of representatives (except for unclassified protein) were the up-regulated signal transduction (with 7.5% of the regulated genes total), transcription factors (6.3%), oxidative burst/hypersensitive response (5.2%), defense response (4.8%) and energy pathways (4.5%) their counterparts found to be down-regulated were much less abundant with 0.4, 2.2, 0.0, 0.7, 0.7% of the regulated genes total, respectively. The category with the larger number of representatives in the down-regulated genes was the photosynthesis related genes with 10 genes suppressed.



(A)



(B)

FIGURE 2 Pie charts showing the number of (A) (up-regulated genes) and (B) (down-regulated genes) in each of the functional categories.

TABLE 2 Classification of gene differentially expressed in *Solanum lycopersicum* 12 hours after NEFID treatment.

Gene classes	Gene number	Response	Ratio (treated/ control)
Up-Regulated = 215 genes – 80% of regulated genes total			
SIGNAL TRANSDUCTION	20	calcium-dependent protein kinase, calmodulin-binding protein, transducin / WD-40 repeat protein , calmodulin, protein kinase, phototropic response protein, phosphatidylinositol-4-phosphate 5-kinase, MAP3K-like protein kinase, mitogen-activated protein kinase kinase (MAPKK), leucine rich repeat protein family contains protein kinase domain, receptor-related serine/threonine kinase, protein phosphatase 2C (PP2C), tyrosine phosphatase, calcineurin-like phosphoesterase, F-box protein family similar to SKP1 interacting partner 2 (SKIP2), transmembrane protein	1.48-2.42
TRANSCRIPTION FACTOR	17	HD-Zip transcription factor Athb-13, bZip DNA binding protein, homeobox-leucine zipper protein HAT5, DHHC-type zinc finger domain, transcription factor L2, MADS-box family protein, homeobox-leucine zipper protein ATHB-13, MADS-box protein 9, MYB family transcription factor, transcription factor SF3, transcriptional factor B3 family protein / auxin-responsive factor, GT-1-related transcription factor, transcriptional adaptor like protein, homeodomain protein contains 'Homeobox' domain signature,	1.40-2.56
OXIDATIVE BURST/HYPERSENSITIVE RESPONSE	14	peroxidase, glutathione peroxidase, copper/zinc superoxide dismutase (CSD2), copper/zinc superoxidase dismutase (CSD1), glyoxalase II, cytochrome P450, glutathione transferase,	1.41-2.62
DEFENSE RESPONSE	13	Pathogenesis-related protein 1 precursor (PR-1) , endo-1,3-beta-glucanase-like protein, basic endochitinase, hevein-related protein precursor (PR-4), pathogenesis-related protein, glycosyl hydrolase family 19 (basic endochitinase), disease resistance protein (NBS-LRR class), leucine rich repeat protein, (PDF2.3) plant defensin protein, disease resistance protein, VIP2 protein, TSW12, Ethylene-responsive proteinase inhibitor I precursor,	1.45-2.88
ENERGY PATHWAYS	12	pyruvate,orthophosphate dikinase, lycopene beta cyclase, malate oxidoreductase (NADP-dependent malic enzyme), gamma-VPE (vacuolar processing enzyme), GCN5-related N-acetyltransferase (GNAT), short-chain dehydrogenase/reductase, mitochondrial aldehyde dehydrogenase (ALDH3), GDP-mannose pyrophosphorylase, L-allo-threonine aldolase, epsilon subunit of mitochondrial F1-ATPase, cytochrome b561-related, cytochrome b5 domain-containing protein	1.47-2.34

… continued…

TABLE 2 Cont.

PROTEIN BIOSYNTHESIS	11	ubiquitin family, deoxyhypusine synthase, cytosolic cyclophilin (ROC3), cyclophilin ROC7, 40S ribosomal protein S14 (RPS14B), 60S ribosomal protein L10A (RPL10aB), 60S acidic ribosomal protein P0 (RPP0B), symbiosis-related like protein, RHO GDP-dissociation inhibitor 1 -related, eukaryotic rpb5 RNA polymerase subunit, eukaryotic translation initiation factor 4A-1 (eIF4A-1)	1.40-1.81
PROTEIN DEGRADATION	9	serine carboxypeptidase, serine carboxypeptidase III, Ethylene-responsive proteinase inhibitor I precursor, proteasome regulatory particle triple-A ATPase subunit4, ubiquitin-conjugating enzyme 2 (UBC2) E2, ubiquitin-associated (UBA)/PB1, ubiquitin-conjugating enzyme 10 (UBC10) E2,	1.54-2.36
CELL WALL	8	endo-1,4-beta-glucanase, cellulase, xyloglucan endotransglycosylase, glycosyltransferase family 8, xyloglucan endo-1,4-beta-D-glucanase (EC 3.2.1.-) precursor, alpha-expansin 6 precursor, Alpha 1,4-glycosyltransferase, O-diphenol-O-methyl transferase,	1.41-2.17
G PROTEIN	5	GTPase activating protein, ARF GTPase-activating domain, GTP-binding protein, Ras-related GTP-binding protein (ARA-4), putative ATP(GTP)-binding protein	1.53-1.87
TRANSPORT	5	ATPase plasma membrane-type (proton pump), H+-transporting ATP synthase-related protein, SNF7 protein, putative UDP-galactose transporter, transporter - related low similarity to hexose transporter	1.46-1.87
CELL STRUCTURE	5	histone H2A, profilin 5, actin polymerisation complex protein, actin-related protein 8B (ARP8) protein	1.51-1.68
NUCLEIC ACID METABOLISM	5	Macrophage migration inhibitory factor (MIF), RNA-binding protein, endonuclease/exonuclease/phosphatase family, ADP-ribosylation factor, RNA recognition motif (RRM)	1.43-2.04
HORMONE RESPONSE	4	auxin-responsive protein, ethylene-response protein ETR1, arginine decarboxylase, 2-oxoglutarate-dependent dioxygenase	1.47-2.59
METABOLISM	4	glutamate decarboxylase (EC 4.1.1.15) 2, starch synthase, phosphomannomutase -related, phytoene synthetase	1.65-2.00
LIPID METABOLISM	4	lipase (class 3) family, ceramidase family protein, myo-inositol-1-phosphate synthase -related protein, thioesterase family	1.55-1.75
STRESS RESPONSE	4	heat shock protein, Pi starvation-induced protein, metallothionein 2b, DnaJ protein	1.43-1.83
PHOTOSYNTHESIS	3	thioredoxin M-type 4, chloroplast precursor (TRX-M4), glutathione synthetase (GSH2), chloroplast nucleoid DNA binding protein,	1.47-1.76
DETOXIFICATION	2	rhodanese-like domain protein	1.53-2.09

… continued …

TABLE 2 Cont.

OTHER	20	steroid sulfotransferase, aldehyde oxidase, 12-oxophytodienoate reductase (OPR3), pescadillo - like protein, oxidoreductase, 2OG-Fe(II) oxygenase, nitrilase 1 like protein, hydrolase alpha/beta fold family, putative spermine/spermidine synthase, heme oxygenase 1 (HO1) gene, COP9 complex subunit, FUS4 FUSCA4, COP8, CSN4, acyltransferase family, fibrillarin 2, oxidoreductase (din11), glutaredoxin protein, iron-sulfur cluster assembly complex protein, hypothetical protein, copper amine oxidase -related, glutaredoxin protein, aldo/keto reductase family, nodulin MtN3 family,	1.44-2.94
UNKNOWN	50	-	1.40-3.76
Down-Regulated = 53 genes – 20% of regulated genes total			
CELL STRUCTURE	3	histone H3, histone H2B, histone H1	0.58-0.60
CELL WALL	2	pectinesterase, N-acetylglucosaminyltransferase	0.38-0.47
DEFENSE RESPONSE	1	terpene synthase/cyclase	0.60
HORMONE RESPONSE	2	ethylene-response protein ETR1, GAST1-related protein induced by gibberellins	0.48-0.60
METABOLISM	1	sugar isomerase	0.55
PROTEIN BIOSYNTHESIS	1	Eukaryotic translation initiation factor 3 subunit 10	0.36
SIGNAL TRANSDUCTION	1	putative membrane protein	0.54
TRANSCRIPTION FACTOR	6	PHD finger transcription factor, lateral organ boundaries (LOB) domain protein 37, ANAC057; transcription factor, GATA zinc finger protein, WRKY family transcription factor	0.41-0.56
TRANSPORT	1	peptide transporter - like protein	0.23
ENERGY PATHWAYS	2	alkaline/neutral invertase, NAD-dependent epimerase/dehydratase	0.49-0.54
LIPID METABOLISM	2	lipoic acid synthase, 3-oxoacyl-[acyl-carrier-protein] synthase I precursor	0.41-0.55
PHOTOSYNTHESIS	10	ribulose bisphosphate carboxylase small chain 3b precursor, ribulose bisphosphate carboxylase small chain 2b precursor, plastocyanin, chlorophyll a-b binding protein 3C-like, light-harvesting chlorophyll a/b binding protein, protochlorophyllide reductase B	0.33-0.59
PROTEINASE INHIBITOR	7	proteinase inhibitor - tomato	0.42-0.48
OTHER	6	putative membrane-associated salt-inducible protein, hypothetical protein DDBDRAFT_0219654, germin-like protein, Tic62 protein, dopamine beta-monooxygenase, gda-1,	0.32-0.57
UNKNOWN	7		0.39-0.55

3.3 NEFID activates the signal transduction in tomato leaves

A total of 20 up-regulated genes were associated with signal transduction, included the induction of six protein kinases (phosphatidylinositol-4-phosphate 5-kinase, MAP3K-like protein kinase, mitogen-activated protein kinase kinase (MAPKK), leucine rich repeat protein family contains protein kinase domain, receptor-related serine/threonine kinase), five genes involved in the calcium signaling such as calcium-dependent protein kinase, calmodulin-binding protein, calmodulin and calcineurin-like phosphoesterase; five phosphatase encoding genes concerned in the dephosphorylation (protein phosphatase 2C (PP2C), tyrosine phosphatase) as well as a transmembrane protein and a transducin/WD-40 repeat protein involved in the GTP mediated signal cascade. In addition, five GTP binding protein (G protein - GTPase activating protein, ARF GTPase-activating domain, GTP-binding protein, Ras-related GTP-binding protein (ARA-4), putative ATP(GTP)-binding protein) and five transport-related genes (ATPase plasma membrane-type (proton pump), H+-transporting ATP synthase-related protein, SNF7 protein, putative UDP-galactose transporter, transporter - related low similarity to hexose transporter) were also up-regulated by the tested formulation.

3.4 NEFID induces hypersensitive reaction and defense response

One of the largest categories of genes regulated by NEFID treatment was the oxidative burst/hypersensitive response-related genes, with 17 genes up-regulated. A substantial proportion of these ESTs have predicted functions in cell rescue and defense processes such as peroxidase, glutathione peroxidase, copper/zinc superoxide dismutase (CSD2), copper/zinc superoxidase dismutase (CSD1), glyoxalase II, cytochrome P450, and glutathione transferase. This category comprises the genes that are responsible for the removal of ROSs.

These induced response support the important role of oxidative burst in activating defense genes.

3.5 NEFID activates PR protein activities

As a result of tomato plant elicitation by NEFID, an up-regulation of genes associated to a putatively known function can undergo post-translational modification and/or silencing which result in a phenotype different from the one inferred from transcriptomics. Thus, additional survey on key pathogenesis-related enzymes was conducted over a time course (0, 24, 48, 72, 96 and 120 hours after spraying) for the activities of peroxidase (POX), glucanase (GLU) and chitinase (CHI) and all were higher than the untreated control as early as 24 hours after spraying (Figure 3). Treatment of tomato plants with NEFID showed a significant ($p \leq 0.05$) increase of POX activity at 24, 48, 72, 96 and 120 HAS. At 48 HAS POX activity demonstrated an abrupt increase in values around fourfold higher than the control, after this peak the POX activity of plants treated with NEFID stayed in values around twofold higher than the control to 120 HAS (Figure 3a).

It was observed that treated plants were able to keep up GLU activity from 24 hours after spraying onward, whereas a tendency GLU activity decrease in water-sprayed control plants compared to the basal level was observed (Figure 3b). At 24-120 HAS interval, GLU activities on treated plants were significantly ($p \leq 0.05$) higher than that of control. At 96 HAS was verified an abrupt peak of GLU activity with values around threefold higher than the control.

Plants sprayed with NEFID, showed a remarkable peak of CHI activity at 24 HAS, twofold higher than the control and from 48-120 HAS and was similar to the control (Figure 3c).

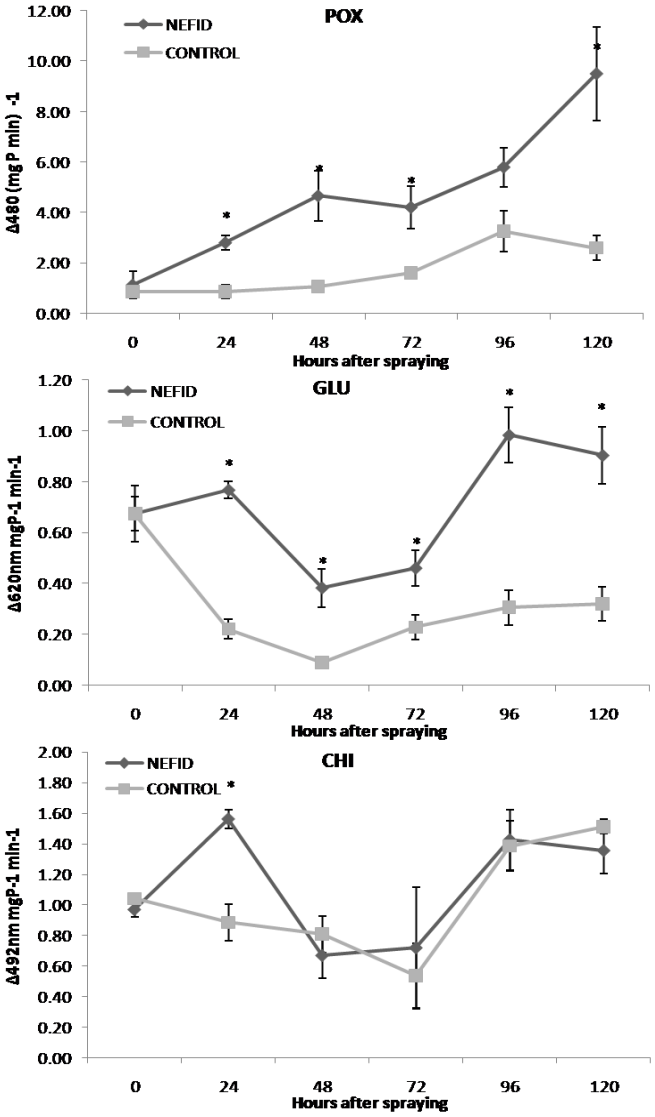


FIGURE 3 Activity of peroxidase (POX) (A), β-1,3-glucanase (GLU) (B), chitinase (CHI) (C) was induced after spraying the tomato seedlings with NEFID in comparison with the water-sprayed control. Enzymatic responses were evaluated 0, 24, 48, 72, 96, 120, hours after spraying. Error bars indicate standard deviations.

4 DISCUSSION

Gene expression profiling through the use of microarrays has been recognized as a powerful approach to obtain overall view on gene expression and physiological processes involved in response to a particular stimulus (Maleck et al., 2000; Schenk et al., 2000). In this study, to better understand underlying physiological events after a coffee leaf plant formulation treatment, we analyzed gene expression profiles of 11,000 tomato genes in tomato leaves at early stage after treatment (12 h) and identified a total of 268 genes that were differentially expressed, which might have been underestimated if the whole genome is considered in future studies since the total estimated tomato genome (35,000 genes) is three fold the number of ESTs used (Van der Hoeven et al., 2002).

From the assigned categories "signal transduction" represents the largest group (9% of up-regulated genes, 2% of down-regulated genes) and pieces of evidence link them (transmembrane ion balance and kinase cascade) to the upstream events underlying the observed defense responses regarding.

Transient changes in the ion permeability of the plasma membrane appear to be a common early event in defense signaling. Upon pathogen recognition, ion channels located in the plasma membrane appear to increase ion fluxes across the membrane and activate downstream defense responses (Wan et al., 2002). Maleck et al. (2000) identified some pump or ion channel genes that were up-regulated by defense-related treatments; for example, a plasma membrane H+-ATPase gene was up-regulated in a constitutive SAR mutants (*cim)* and up-regulated in systemic leaves expressing *avrRpt2* after challenging them with *P. syringae* pv. *tomato* DC3000. A rapid expression change as were observed for signal transduction, GTP binding protein and transport-related genes is a possible direct consequence of NEFID-mediated

plasma membrane ATPase (proton pump) activation, which may lead PR gene expression.

Another link between signal transduction and down-stream PR activation is related to the mithogen activated kinases (MAP3K and MAPKK) and calcium dependent kinases (calmodulin and calcium protein kinases) as well as the absence of evidence for salicylic or jasmonic acid/ethylene metabolisms suggesting for a kinase cascade signaling leading to a SAR-independent pathogenesis-related protein activation as previously shown for fusicoccin-mediated PR accumulation in tomato (Frick & Schaller, 2002).

Furthermore, the down-regulation of ethylene response protein and terpene synthase/cyclase (jasmic acid biosynthesis) suggests that the observed disease resistance is not likely to have a consequence on fruit ripening. The constraint was pointed out by Jiang et al. (2009) studying post harvest disease resistance induction in cherry tomato. They found that in spite of the up-regulation of PRs in a JA-independent manner, an up-regulation of both jasmonic acid biosynthesis and ethylene receptors were up-regulated.

Another possible side effect related to induced resistance is the shift in the primary metabolism to provide backbone carbon for resistance proteins and related molecules such as glutamate decarboxylase and lipase (Shah, 2005) as well as a down-regulation of photosynthesis-related genes. The photosynthesis is not likely to be linked to the jasmonate-mediated inhibition of photosynthetic pigments and photosynthetic activity (Frick & Schaller, 2002) and measurements of photosynthesis at a later time point (8d after spray) showed no difference between NEFID-treated and control plants (Fig. 4), suggesting for a post-transcriptional regulation or silencing regulation of those genes.

4.1 Oxidative burst and hypersensitive reaction

The “oxidative burst/hypersensitive response” is among the largest group of genes with changed regulation after treatment with NEFID formulation (7% of up-regulated ones). Signaling compounds production such as reactive oxygen species (ROSs) causes activation of many downstream responses (Khan & Wilson, 1995). Increasing verification has suggested that ROSs play a role directly as antimicrobial compounds, and also as signaling molecules in plant defense. ROSs are important in activating defense gene expression in adjacent cells and the whole plant, probably in combination with other signaling molecules (Buchanan, 2000).

Because reactive oxygen species (ROS) can cause damage to proteins, lipids and DNA, ROS production and removal must be strictly controlled. Various ROS-scavenging systems, such as glutathione, superoxide dismutases, catalases and ascorbate peroxidases, maintain ROS homeostasis in different compartments of the plant cell (Mittler et al., 2004). These enzymes could restrict the ROS-dependent damage or finely tune ROS-dependent signal transduction (Torres et al., 2006). Differential regulation of these enzymes, in part mediated by SA, may contribute to increases in ROS and activation of defenses following infection (Dorey et al., 1998; Mittler et al., 1999; Klessig et al., 2000).

4.2 Defense-related

NEFID-induced expression changes of potential defense responses genes. Most of the up-regulated responses in defense-related transcripts have been reported as part of the salicylic acid dependent pathway (PR-1, lipid transfer protein TSW12, basic endochitinase, hevein-related protein precursor (PR-4), VIP2 protein, endo-1,4-beta-glucanase, disease resistance protein (NBS-LRR class), glucan endo-1,3-beta-glucosidase) except (PDF2.3) plant

defensin which is putatively a product of the jasmonate/ethylene pathway (Pieterse & van Loon, 1999). Conversely, the gene coding for terpene synthase/cyclase, which is involved in methyl jasmonate pathway biosynthesis (Martin et al., 2003), was down-regulated in NEFID-treated plants.

A marker gene of systemic acquired resistance (PR-1) is generally associated to the salicylic acid pathway but in this case no evidence of SA accumulation was found and future studies will monitor salicylic acid in different time points in order to determine if SA plays a role in upstream signaling of the observed PRs. However, the PR translation triggering has already been mentioned as SA-independent (Frick &Schaller, 2002).

Various are the roles of the observed PRs acting either directly on the pathogen or indirectly by creating physical barriers to the fungal infection process or upstream intrinsic PR signaling.

Most of the found PRs act directly on the disruption of the fungal/bacterial cell wall (endo-1,4-beta-glucanase, basic endochitinase and glucan endo-1,3-beta-glucosidase), or inhibiting the fungus germination due to the ribonuclease activity (hevein-like precursor) (Datta & Muthukrishnan, 1999; Caporale et al., 2004). And the assessment of the enzyme activities (glucanase and chitinase) (Fig. 3) confirms the translation of the protein as well as their long-lasting expression particularly glucanase.

Also found to be up-regulated was a defensin (PDF1.2), a gene putatively associated to the jasmonate-dependent pathway. Since no gene peculiar to this pathway was found, future experiments will determine the upstream signaling leading to the expression of this protein (Pieterse & van Loon, 1999).

In an indirect way, the lipid transfer protein TSW12 is referred to as cuticle formation, an indirect protection against pathogen invasion as well as drought tolerance (Torres-Schumann et al., 1992; Molina & Garcia-Olmedo,

1997). As early events of the defense signaling, the disease resistance protein (NBS-LRR class) plays a role in fast signal transduction (Belkhadir, 2004).

4.3 Diverse possible roles of the NEFID-mediated changes

Apart from the PRs, the cytochrome P450, found to be up-regulated, has been reported in the production of antimicrobial substances (Jiang et al., 2009) and may play an additional role in the defense against pathogens.

Since plants haven't been challenged with any pathogen, which possibly fine tunes the plant metabolism for the defense responses (Guzzo et al., 2009), broad responses were observed such as the possible up-regulation of genes involved in stress alleviation in regard to reactive oxygen species (glutathione S-transferase, peroxidase) or temperature (heat shock protein).

4.4 Traits with unknown role on defense responses

The "transcription factor" represents the largest number of genes in both up and down regulation (8% of up-regulated genes, 11% of down-regulated genes) but the direct relationship on disease resistance needs further evidence.

Similar to the mentioned category, the role of other categories not detailed in this study will be the focus of future experiments.

In previous works, the effectiveness of the rust infected coffee leaf formulation has been demonstrated in the control of either fungal or bacterial diseases in coffee or cotton (Santos et al., 2007; Ishida et al., 2007), previous work has also demonstrated its potential in the control of bacterial spot as well as the commercial elicitor acibenzolar-S-methyl (data not presented) and in the study we chose not to inoculate plants to have an insight of the broad range of possible targets of the NEFID-mediated disease resistance and used tomato as the model plant. Potentially responses to bacterial (glucanase/lisozyme and

defensin) and fungal (chitinase, glucanase and hevein) have been identified, not impossible that other pathogens may be affected by the afore-mentioned PRs such as nematodes and virus.

Nematode eggs are made up of chitin and chitinase has already been implicated in disruption of egg shells leading to a reduction in hatching (Qiu et al., 1997). A more complicated case is the induction of resistance against virus. A piece of evidence suggests that chaperone interact with the movement protein of the virus hindering its cell-to-cell movement (Von Bargen et al., 2001).

A fungal cell derivative (chitosan), such as the one likely to be present in the used infected leaf extract was found to induce the expression of DNAJ-related chaperone, also found as up-regulated in our studies, similar to the one found in the control of TSWV in tomato, tobacco and *Arabidopsis* (Zhang et al., 2007). The same elicitor has proven to control the root knot nematode *Meloidogyne incognita*, showing a reduction in number of eggs and fertile females and the mode of action was the activation of PRs, notably chitinase (Zinov'eva et al., 2001).

Finally, the tested plant disease elicitor, NEFID, has shown to induce multiple plant defense responses with the potential to be used against multiple pathogens. The induction does not interfere in the photosynthesis and the pathogenesis-related proteins are not likely to suffer post-tranlational regulation since the glucanase, peroxidase and chitinase activities were found to be over-expressed as early as 24h after spray and eventually last for at least five days (glucanse and peroxidase) at high levels, suggesting for a long lasting effect of elicitation.

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GENERAL CONCLUSIONS

Tomato plants treated with a natural compost of coffee leaf (NEFID) demonstrated 35% of reduction to the bacterial spot severity, a significatively reduction (61%) was obtained by ASM-treated plants.

The severity reduction of the bacterial spot disease appears to be based on a premature β-1,3-glucanase and PR-1 gene expression increase (12 hours after spraying) followed by an early response in a chitinase, glucanase and polyphenol oxidase activity (24 hours after spraying) and a earlier high lignin deposition on leaf tissue.

A natural compost of coffee leaf (NEFID) has the potential to activate defense related genes and they can be monitored using microarray technology.

A total of 268 genes had changed regulation with a majority of up-regulated ones which encoded mainly signal transduction, defense –related, oxidative burst and transcription factor, when tomato plants are sprayed with the NEFID.

Since no evidence for salicylic acid or jasmonic acid was found but mitogen activated proteins (MAP3K and MAPKK) as well as calcium dependent (calmodulin and phosphatidylinositol) signaling molecules were found, a SA-independent PR accumulation is likely to occur.

The PRs chitinase, glucanase and hevein-like, with direct reported activity on pathogens, were up-regulated with the application of NEFID on tomato plants, and they are not likely to suffer post-translational regulation since the corresponding enzyme activities were over-expressed as early as 24h after treatment and eventually remained as such for up to five days onward (glucanase and peroxidase).

The studied plant formulation represent a potential broad spectrum disease control.

ANEXES

PAGE

TABLE 1 NEFID-responsive tomato genes identified by microarray approaches.

ID	Gene Annotation	Average of ratios	Pvalue
	Up-Regulated		
	NUCLEIC ACID METABOLISM		
M84744	Tomato phytoene synthetase mRNA, complete cds	1.49	0.01
SGN-U212772	arabidopsis/peptide: At3g62290.1 68410.m06466 ADP-ribosylation factor identical to GP:166586 ADP-ribosylation factor {Arabidopsis thaliana}; ADP-ribosylation factor 1 - Arabidopsis thaliana, PIR:S28875 (evalue: 3e-100, score=361.3) genbank/nr: gi|1703380|sp|P51823|ARF_ORYSA ADP-ribosylation factor gi|25294170|pir||T52341 ADP-ribosylation factor [imported] - rice gi|1132483|dbj|BAA04607.1| ADP-ribosylation factor [Oryza sativa (japonica cultivar-group)] gi|13646976|dbj|BAB41081.1| ADP-ribosylation factor [Triticum aestivum] gi|23304413|emb|CAD48129.2| ADP-ribosylation factor 1-like protein [Hordeum vulgare subsp. vulgare] (evalue: 8e-100, score=365.2)	1.79	0.04
SGN-U214340	arabidopsis/peptide: At3g19130.1 68410.m02193 RNA-binding protein, putative similar to RNA Binding Protein 47 [Nicotiana plumbaginifolia] GI:9663769, DNA binding protein ACBF GB:AAC49850 from [Nicotiana tabacum]; contains InterPro entry IPR000504: RNA-binding region RNP-1 (RNA recognition motif) (RRM) (evalue: 2e-125, score=446) genbank/nr: gi|7489196|pir||T01932 RNA binding protein homolog - common tobacco (fragment) gi|2708532|gb|AAB92518.1| putative RNA binding protein [Nicotiana tabacum] (evalue: 9e-164, score=578.9)	1.44	0.08
SGN-U215885	arabidopsis/peptide: At1g47510.1 68408.m04833 endonuclease/exonuclease/phosphatase family similar to SP|P32019 Type II inositol-1,4,5-trisphosphate 5-phosphatase precursor (EC 3.1.3.56) {Homo sapiens}; contains Pfam profile PF03372: Endonuclease/Exonuclease/phosphatase family (evalue: 2e-108, score=389) genbank/nr: gi|37718787|gb|AAR01658.1| putative inositol polyphosphate 5-phosphatase [Oryza sativa (japonica cultivar-group)] (evalue: 7e-112, score=406)	1.83	0.03
SGN-U227378	arabidopsis/peptide: At3g45630.1 68410.m04565 RNA recognition motif (RRM) - containing protein similar to SP|P34909 General negative regulator of transcription subunit 4 {Saccharomyces cerevisiae}; contains InterPro entry IPR000504: RNA-binding region RNP-1 (RNA recognition motif) (RRM) (evalue: 2.5e-96, score=348.2) genbank/nr: gi|15231193|ref|NP_190149.1| RNA recognition motif (RRM)-containing protein [Arabidopsis thaliana] gi|11357925|pir||T47503 hypothetical protein F9K21.210 - Arabidopsis thaliana gi|6996266|emb|CAB75492.1| putative protein [Arabidopsis thaliana] (evalue: 8.9e-95, score=348.2)	2.05	0.04

… continued…

TABLE 1 Cont.

	CELL STRUCTURE		
SGN-U213866	arabidopsis/peptide: At1g51060.1 68408.m05267 histone H2A, putative similar to histone H2A GI:7595337 from Arabidopsis thaliana, Triticum aestivum GI:536892, Picea abies SP|P35063; contains Pfam profile PF00125 Core histone H2A/H2B/H3/H4 (evalue: 6.1e-46, score=181) genbank/nr: gi|15223708|ref|NP_175517.1| histone H2A, putative [Arabidopsis thaliana] gi|25294262|pir||G96547 probable histone H2A [imported] - Arabidopsis thaliana gi|12320785|gb|AAG50540.1| histone H2A, putative [Arabidopsis thaliana] gi|13877851|gb|AAK44003.1| putative histone H2A protein [Arabidopsis thaliana] gi|17065594|gb|AAL33777.1| putative histone H2A protein [Arabidopsis thaliana] (evalue: 2.2e-44, score=181)	1.51	0.04
SGN-U214142	arabidopsis/peptide: At5g56600.1 68412.m06396 profilin 5 (evalue: 2.1e-48, score=189.1) genbank/nr: gi|16555787|emb|CAD10377.1| profilin [Lycopersicon esculentum] (evalue: 1.4e-61, score=238)	2.14	0.04
SGN-U215974	arabidopsis/peptide: At5g56180.1 68412.m06343 actin-related protein 8B (ARP8) protein annotation temporarily based on supporting cDNA gi|21427470|gb|AF507916.1| (evalue: 0, score=634) genbank/nr: gi|30696705|ref|NP_568836.2| actin-related protein 8B (ARP8) protein [Arabidopsis thaliana] gi|9758638|dbj|BAB09300.1| contains similarity to actin~gene_id:MDA7.24 [Arabidopsis thaliana] gi|21427471|gb|AAM53248.1| actin-related protein 8B [Arabidopsis thaliana] gi|21489926|tpg|DAA00031.1| TPA: actin-related protein 8B; AtARP8B [Arabidopsis thaliana] (evalue: 3e-180, score=634)	1.68	0.07
SGN-U217962	arabidopsis/peptide: At1g51060.1 68408.m05267 histone H2A, putative similar to histone H2A GI:7595337 from Arabidopsis thaliana, Triticum aestivum GI:536892, Picea abies SP|P35063; contains Pfam profile PF00125 Core histone H2A/H2B/H3/H4 (evalue: 4.1e-32, score=134.8) genbank/nr: gi|15223708|ref|NP_175517.1| histone H2A, putative [Arabidopsis thaliana] gi|25294262|pir||G96547 probable histone H2A [imported] - Arabidopsis thaliana gi|12320785|gb|AAG50540.1| histone H2A, putative [Arabidopsis thaliana] gi|13877851|gb|AAK44003.1| putative histone H2A protein [Arabidopsis thaliana] gi|17065594|gb|AAL33777.1| putative histone H2A protein [Arabidopsis thaliana] (evalue: 1.4e-30, score=134.8)	1.56	0.01
SGN-U219052	arabidopsis/peptide: At1g60430.1 68408.m06240 expressed protein similar to putative actin polymerisation complex protein GI:4539247 from [Schizosaccharomyces pombe] (evalue: 5e-85, score=311.2) genbank/nr: gi|21536845|gb|AAM61177.1| Contains similarity to 21 KD subunit of the Arp2/3 protein complex (ARC21) [Arabidopsis thaliana] (evalue: 1.9e-83, score=311.2)	1.62	0.08
	CELL WALL		

… continued …

TABLE 1 Cont.

SGN-U213455	arabidopsis/peptide: At5g57560.1 68412.m06509 xyloglucan endotransglycosylase (TCH4) identical to xyloglucan endotransglycosylase TCH4 protein GI:886116 (evalue: 1e-101, score=366.7) genbank/nr: gi|1076604|pir||S49812 xyloglucan endo-1,4-beta-D-glucanase (EC 3.2.1.-) precursor (clone tXET-B1) - tomato gi|577066|emb|CAA58003.1| xyloglucan endo-transglycosylase [Lycopersicon esculentum] (evalue: 7e-153, score=542.3)	2.18	0.03
SGN-U218121	arabidopsis/peptide: At1g64390.1 68408.m06687 glycosyl hydrolase family 9 (endo-1,4-beta-glucanase) similar to endo-beta-1,4-glucanase GI:4972236 from [Fragaria x ananassa] (Plant Mol. Biol. 40, 323-332 (1999)) (evalue: 0, score=803.9) genbank/nr: gi|4165132|gb|AAD08699.1| endo-beta-1,4-D-glucanase [Lycopersicon esculentum] (evalue: 0, score=995.3)	1.42	0.02
SGN-U214839	arabidopsis/peptide: At5g49720.1 68412.m05554 glycosyl hydrolase family 9 (endo-1,4-beta-glucanase) similar to endo-1,4-beta-D-glucanase; cellulase GI:5689613 from [Brassica napus] (evalue: 0, score=950.7) genbank/nr: gi|7488979|pir||T07612 cellulase (EC 3.2.1.4) Cel3, membrane-anchored - tomato gi|2065531|gb|AAC49704.1| endo-1,4-beta-glucanase [Lycopersicon esculentum] (evalue: 0, score=1072.8)	1.71	0.01
SGN-U215711	arabidopsis/peptide: At2g39700.1 68409.m04413 expansin, putative (EXP4) similar to alpha-expansin 6 precursor GI:16923359 from [Cucumis sativus]; alpha-expansin gene family, PMID:11641069 (evalue: 7e-124, score=440.7) genbank/nr: gi|7488994|pir||T07630 expansin 1 - tomato gi|2062421|gb|AAC63088.1| expansin [Lycopersicon esculentum] gi|33334359|gb|AAQ12264.1| expansin 1 protein; LeExp1 [Lycopersicon esculentum] (evalue: 3e-152, score=540)	1.82	0.08
SGN-U216932	arabidopsis/peptide: At3g53140.1 68410.m05423 O-diphenol-O-methyl transferase, putative similar to GI:6688808 [Medicago sativa subsp. x varia], caffeic acid O-methyltransferase (homt1), Populus kitakamiensis, EMBL:PKHOMT1A (evalue: 7e-140, score=493.8) genbank/nr: gi|15231756|ref|NP_190882.1| O-diphenol-O-methyl transferase, putative [Arabidopsis thaliana] gi|11279303|pir||T46160 caffeic acid O-methyltransferase-like protein - Arabidopsis thaliana gi|6630734|emb|CAB64217.1| caffeic acid O-methyltransferase-like protein [Arabidopsis thaliana] gi|14194165|gb|AAK56277.1| AT3g53140/T4D2_70 [Arabidopsis thaliana] gi|22137206|gb|AAM91448.1| AT3g53140/T4D2_70 [Arabidopsis thaliana] (evalue: 3e-138, score=493.8)	1.46	0.04
SGN-U218042	arabidopsis/peptide: At1g11545.1 68408.m01194 xyloglucan endotransglycosylase, putative similar to endo-xyloglucan transferase GI:2244732 from [Gossypium hirsutum] (evalue: 4.7e-85, score=243.4) genbank/nr: gi|18391291|ref|NP_563892.1| xyloglucan endotransglycosylase, putative [Arabidopsis thaliana] (evalue: 1.7e-83, score=243.4)	1.85	0.06

… continued …

TABLE 1 Cont.

SGN-U220366	arabidopsis/peptide: At3g58790.1 68410.m06057 glycosyltransferase family 8 contains Pfam profile: PF01501 glycosyl transferase family 8; general stress protein gspA, Bacillus subtilis, PIR:S16423 (evalue: 2e-162, score=569.3) genbank/nr: gi|22331857|ref|NP_191438.2| glycosyltransferase family 8 [Arabidopsis thaliana] gi|20466464|gb|AAM20549.1| putative protein [Arabidopsis thaliana] gi|22136432|gb|AAM91294.1| putative protein [Arabidopsis thaliana] (evalue: 9e-161, score=569.3)	1.55	0.09
SGN-U220698	arabidopsis/peptide: At4g19900.1 68411.m02661 glycosyltransferase-related contains Pfam profiles PF01535: PPR repeat, PF04572: Alpha 1,4-glycosyltransferase conserved region, PF04488: Glycosyltransferase sugar-binding region containing DXD motif; several hypothetical proteins - Arabidopsis thaliana (evalue: 5.2e-27, score=117.9) genbank/nr: gi|34899808|ref|NP_911250.1| OJ1092_A07.13 [Oryza sativa (japonica cultivar-group)] gi|27817893|dbj|BAC55659.1| unknown protein [Oryza sativa (japonica cultivar-group)] (evalue: 5.6e-27, score=122.9)	1.55	0.01
	DEFENSE RESPONSE		
SGN-U212883	arabidopsis/peptide: At3g12500.1 68410.m01395 glycosyl hydrolase family 19 (basic endochitinase) identical to basic endochitinase precursor SP:P19171 from [Arabidopsis thaliana] (evalue: 1e-123, score=439.9) genbank/nr: gi|544011|sp|Q05538|CHIC_LYCES Basic 30 kDa endochitinase precursor gi|487033|pir||S37344 chitinase (EC 3.2.1.14) chi9 precursor - tomato gi|19191|emb|CAA78845.1| chitinase [Lycopersicon esculentum] (evalue: 7e-169, score=595.5)	1.65	0.08
SGN-U212922	arabidopsis/peptide: At2g14580.1 68409.m01475 pathogenesis-related protein, putative similar to SP|P33154 Pathogenesis-related protein 1 precursor (PR-1) {Arabidopsis thaliana}; contains Pfam profile PF00188: SCP-like extracellular protein (evalue: 1.8e-49, score=192.6) genbank/nr: gi|548587|sp|P04284|PR06_LYCES Pathogenesis-related leaf protein 6 precursor (P6) (Ethylene-induced protein P1) (P14) (P14A) (PR protein) gi|2144919|pir||VCTO14 pathogenesis-related protein P6 precursor - tomato gi|19285|emb|CAA48672.1| P1(p14) protein [Lycopersicon esculentum] gi|170490|gb|AAA03616.1| pathogenesis-related protein P6 gi|2529165|emb|CAA70042.1| PR protein [Lycopersicon esculentum] (evalue: 4.7e-94, score=345.9)	1.58	0.02
SGN-U212923	arabidopsis/peptide: At4g33720.1 68411.m04354 pathogenesis-related protein, putative similar to SP|P33154 Pathogenesis-related protein 1 precursor (PR-1) {Arabidopsis thaliana}; contains Pfam profile PF00188: SCP-like extracellular protein (evalue: 3.8e-51, score=198.7) genbank/nr: gi|548586|sp|Q04108|PR04_LYCES Pathogenesis-related leaf protein 4 precursor (P4) gi|2119761|pir||S26238 pathogenesis-related protein isoform P4 precursor - tomato gi|170488|gb|AAA03615.1| pathogenesis-related protein P4 gi|3660529|emb|CAA09671.1| pathogenesis-related protein PR1a (P4) [Lycopersicon esculentum] (evalue: 3.3e-94, score=347.1)	1.84	0.01

… continued …

TABLE 1 Cont.

SGN-U212943	arabidopsis/peptide: At3g46570.1 68410.m04684 glycosyl hydrolase family 17 similar to glucan endo-1,3-beta-glucosidase precursor SP:P52409 from [Triticum aestivum] (evalue: 1.5e-20, score=96.29) genbank/nr: gi|13548679|dbj|BAB40807.1| endo-1,3-beta-glucanase-like protein [Pyrus pyrifolia] (evalue: 6.1e-23, score=109.4)	1.61	0.02
SGN-U213613	arabidopsis/peptide: At5g43580.1 68412.m04803 hypothetical protein (evalue: 3.6e-08, score=54.3) genbank/nr: gi|124192|sp|P20076|IER1_LYCES Ethylene-responsive proteinase inhibitor I precursor gi|82085|pir||A32067 ethylene-responsive proproteinase inhibitor I precursor - tomato gi|623594|gb|AAA60745.1| proteinase inhibitor I (evalue: 1e-09, score=64.31)	2.89	0.09
SGN-U214103	arabidopsis/peptide: At5g61240.1 68412.m06950 leucine rich repeat protein family contains leucine rich-repeat (LRR) domains Pfam:PF00560, INTERPRO:IPR001611; contains similarity to Hcr2-0B [Lycopersicon esculentum] gi|3894387|gb|AAC78593 (evalue: 1e-131, score=466.8) genbank/nr: gi|14626935|gb|AAK70805.1| leucine-rich repeat resistance protein-like protein [Gossypium hirsutum] (evalue: 2e-140, score=501.5)	1.48	0.03
SGN-U214589	arabidopsis/peptide: At2g02130.1 68409.m00129 plant defensin protein, putative (PDF2.3) plant defensin protein family member, personal communication, Bart Thomma (Bart.Thomma@agr.kuleuven.ac.be) (evalue: 5.8e-09, score=57.38) genbank/nr: gi|4753797|emb|CAB42006.1| gamma-thionin [Lycopersicon esculentum] (evalue: 2.5e-15, score=83.57)	1.58	0.09
SGN-U214985	arabidopsis/peptide: At3g04720.1 68410.m00468 hevein-related protein precursor (PR-4) identical to hevein-like protein precursor GB:P43082 [Arabidopsis thaliana], similar to wound-induced protein (WIN2) precursor GB:P09762 [Solanum tuberosum]; Pfam HMM hit: chitin_binding proteins (evalue: 7.3e-51, score=196.8) genbank/nr: gi|400851|sp|P32045|PRP2_LYCES Pathogenesis-related protein P2 precursor gi|100232|pir||S23801 pathogenesis-related protein P2 precursor - tomato gi|19976|emb|CAA41439.1| pathogenesis-related protein P2 [Lycopersicon esculentum] (evalue: 1e-82, score=307.8)	1.68	0.06
SGN-U215247	arabidopsis/peptide: At5g59710.1 68412.m06775 VIP2 protein annotation temporarily based on supporting cDNA gi|12006938|gb|AF295433.1|AF295433 (evalue: 2.7e-72, score=269.2) genbank/nr: gi|25406997|pir||B86212 protein F24B9.20 [imported] - Arabidopsis thaliana gi|8439898|gb|AAF75084.1| F24B9.20 [Arabidopsis thaliana] (evalue: 3.5e-79, score=297.4)	1.86	0.08
SGN-U224778	arabidopsis/peptide: At3g12500.1 68410.m01395 glycosyl hydrolase family 19 (basic endochitinase) identical to basic endochitinase precursor SP:P19171 from [Arabidopsis thaliana] (evalue: 1.9e-10, score=63.16) genbank/nr: gi|46396546|sp|Q9S8M0|LECT_SOLTU Chitin-binding lectin 1 precursor (PL-I) (evalue: 8.2e-42, score=172.6)	2.03	0.06

… continued …

TABLE 1 Cont.

SGN-U225938	arabidopsis/peptide: At2g25470.1 68409.m02764 leucine rich repeat protein family contains leucine rich-repeat (LRR) domains Pfam:PF00560, INTERPRO:IPR001611; contains similarity to disease resistance protein [Lycopersicon esculentum] gi|3894383|gb|AAC78591 (evalue: 2.5e-10, score=62.77) genbank/nr: gi|44888781|gb|AAS48162.1| LRR protein WM1.2 [Aegilops tauschii] (evalue: 8.4e-15, score=82.8)	1.67	0.02
SGN-U227625	arabidopsis/peptide: At3g14460.1 68410.m01642 disease resistance protein (NBS-LRR class), putative domain signature NBS-LRR exists, suggestive of a disease resistance protein (evalue: 9.6e-25, score=110.2) genbank/nr: gi|32470648|gb|AAP45174.1| putative disease resistant protein rga4 [Solanum bulbocastanum] (evalue: 2e-114, score=413.3)	1.45	0.09
X56040	L.esculentum TSW12 mRNA	2.13	0.03
	DETOXIFICATION		
SGN-U219160	arabidopsis/peptide: At4g24750.1 68411.m03227 expressed protein (evalue: 5e-72, score=268.5) genbank/nr: gi|46805304|dbj|BAD16836.1| rhodanese-like domain-containing protein -like [Oryza sativa (japonica cultivar-group)] >gi|47847819|dbj|BAD21614.1| rhodanese-like domain-containing protein -like [Oryza sativa (japonica cultivar-group)] (evalue: 1.9e-78, score=295)	2.1	0
SGN-U220749	arabidopsis/peptide: At3g08920.1 68410.m00934 rhodanese-like domain protein contains rhodanese-like domain PF:00581 (evalue: 4.5e-68, score=254.6) genbank/nr: gi|18491227|gb|AAL69515.1| putative rhodanese family protein [Arabidopsis thaliana] (evalue: 1.7e-66, score=254.6)	1.53	0.03
	ENERGY PATHWAYS		
SGN-U213052	arabidopsis/peptide: At1g79750.1 68408.m08532 malate oxidoreductase -related similar to malate oxidoreductase (NADP-dependent malic enzyme) GB:P34105 (Populus balsamifera subsp. trichocarpa) (evalue: 0, score=918.3) genbank/nr: gi|7431232|pir||T06402 malate dehydrogenase (oxaloacetate-decarboxylating) (NADP) (EC 1.1.1.40) 2, cytosolic - tomato gi|2150029|gb|AAB58728.1| cytosolic NADP-malic enzyme [Lycopersicon esculentum] (evalue: 0, score=1133.2)	2.03	0.01
SGN-U213366	arabidopsis/peptide: At2g39770.1 68409.m04425 GDP-mannose pyrophosphorylase identical to GDP-mannose pyrophosphorylase from Arabidopsis thaliana [GI:3598958]; updated per Conklin PL et al, PNAS 1999, 96(7):4198-203 (evalue: 0, score=654.8) genbank/nr: gi|47681456|gb|AAT37498.1| GDP-mannose pyrophosphorylase [Lycopersicon esculentum] (evalue: 0, score=718.4)	1.54	0.06

… continued …

TABLE 1 Cont.

SGN-U214009	arabidopsis/peptide: At1g23800.1 68408.m02717 mitochondrial aldehyde dehydrogenase (ALDH3) similar to mitochondrial aldehyde dehydrogenase ALDH3[Arabidopsis thaliana] gi|19850249|gb|AAL99612 (evalue: 3e-102, score=367.9) genbank/nr: gi|20530131|dbj|BAB92019.1| mitochondrial aldehyde dehydrogenase [Sorghum bicolor] (evalue: 3e-105, score=383.3)	1.98	0.04
SGN-U214299	arabidopsis/peptide: At3g46170.1 68410.m04627 short-chain dehydrogenase/reductase family protein contains similarity to 3-oxoacyl-[acyl-carrier protein] reductase SP:P51831 from [Bacillus subtilis] (evalue: 7e-46, score=126.3) genbank/nr: gi|15231362|ref|NP_190203.1| short-chain dehydrogenase/reductase family protein [Arabidopsis thaliana] gi|11250534|pir||T49258 dehydrogenase-like protein - Arabidopsis thaliana gi|7799005|emb|CAB90944.1| dehydrogenase-like protein [Arabidopsis thaliana] (evalue: 2.4e-44, score=137.1)	1.52	0.04
SGN-U215938	arabidopsis/peptide: At4g15530.1 68411.m02150 pyruvate,orthophosphate dikinase (evalue: 0, score=867.5) genbank/nr: gi|3024426|sp|Q42910|PODK_MESCR Pyruvate,phosphate dikinase, chloroplast precursor (Pyruvate,orthophosphate dikinase) gi|1084302|pir||S55478 pyruvate, phosphate dikinase (EC 2.7.9.1) - common ice plant gi|854265|emb|CAA57872.1| pyruvate,orthophosphate dikinase [Mesembryanthemum crystallinum] (evalue: 0, score=902.5)	1.98	0.04
SGN-U216270	arabidopsis/peptide: At4g16820.1 68411.m02311 lipase (class 3) family similar to DEFECTIVE IN ANTHER DEHISCENCE1 [Arabidopsis thaliana] GI:16215706; contains Pfam profile PF01764: Lipase (evalue: 1.6e-71, score=266.2) genbank/nr: gi|18414755|ref|NP_567515.1| lipase (class 3) family [Arabidopsis thaliana] (evalue: 6.1e-70, score=266.2)	1.54	0.07
SGN-U216555	arabidopsis/peptide: At1g51650.1 68408.m05341 epsilon subunit of mitochondrial F1-ATPase identical to epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] GI:1655486 (evalue: 2.9e-28, score=121.7) genbank/nr: gi|15217996|ref|NP_175576.1| epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] gi|2493052|sp|Q96253|ATP5_ARATH ATP synthase epsilon chain, mitochondrial gi|25290492|pir||C96555 protein epsilon subunit of mitochondrial F1-ATPase [imported] - Arabidopsis thaliana gi|1655486|dbj|BAA13602.1| epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] gi|12321688|gb|AAG50890.1| epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] gi|18252167|gb|AAL61916.1| epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] gi|21386911|gb|AAM47859.1| epsilon subunit of mitochondrial F1-ATPase [Arabidopsis thaliana] (evalue: 9.9e-27, score=121.7)	1.52	0

… continued …

TABLE 1 Cont.

SGN-U226422	arabidopsis/peptide: At2g43130.1 68409.m04843 Ras-related GTP-binding protein (ARA-4) identical to SP:P28187 from [Arabidopsis thaliana] (evalue: 8.8e-20, score=92.82) genbank/nr: gi|7438428|pir||T06443 GTP-binding protein - garden pea gi|303730|dbj|BAA02108.1| GTP-binding protein [Pisum sativum] gi|738933|prf||2001457A GTP-binding protein (evalue: 1.1e-18, score=93.97)	1.84	0.07
	HORMONE RESPONSE		
L16582	Lycopersicon esculentum arginine decarboxylase mRNA, complete cds	1.48	0.04
SGN-U212804	arabidopsis/peptide: At1g06620.1 68408.m00635 2-oxoglutarate-dependent dioxygenase, putative similar to 2A6 (GI:599622) and tomato ethylene synthesis regulatory protein E8 (SP|P10967); contains Pfam profile: PF00671 Iron/Ascorbate oxidoreductase family (evalue: 1e-106, score=383.6) genbank/nr: gi|119640|sp|P10967|ACC3_LYCES 1-aminocyclopropane-1-carboxylate oxidase homolog (Protein E8) gi|82109|pir||S01642 ripening protein E8 - tomato gi|19199|emb|CAA31789.1| E8 protein [Lycopersicon esculentum] (evalue: 0, score=718.8)	2.6	0.02
SGN-U214005	arabidopsis/peptide: At1g66340.1 68408.m06909 ethylene-response protein, ETR1 identical to GB:P49333 from [Arabidopsis thaliana] (Science 262 (5133), 539-544 (1993)) (evalue: 7.9e-11, score=64.31) genbank/nr: gi|7488991|pir||T07026 ethylene receptor - tomato (strain Ailsa Craig) (fragment) gi|984157|emb|CAA90808.1| ethylene receptor [Lycopersicon esculentum] (evalue: 1.3e-33, score=145.2)	1.53	0.07
SGN-U222167	arabidopsis/peptide: At2g46370.2 68409.m05709 auxin-responsive protein family similar to auxin-responsive GH3 product [Glycine max] GI:18591; contains Pfam profile PF03321: GH3 auxin-responsive promoter (evalue: 0, score=636.3) genbank/nr: gi|48843811|gb|AAT47070.1| putative auxin-regulated protein [Oryza sativa (japonica cultivar-group)] (evalue: 0, score=636.3)	1.79	0.09
	LIPID METABOLISM		
SGN-U213442	arabidopsis/peptide: At5g10170.1 68412.m01086 myo-inositol-1-phosphate synthase -related protein myo-inositol-1-phosphate synthase, Nicotiana paniculata, EMBL:AB032073 (evalue: 0, score=916) genbank/nr: gi|14548096|sp|Q9LW96|INO1_TOBAC Inositol-3-phosphate synthase (Myo-inositol-1-phosphate synthase) (MI-1-P synthase) (IPS) gi|8096266|dbj|BAA95788.1| myo-inositol 1-phosphate synthase [Nicotiana tabacum] (evalue: 0, score=974.5)	1.72	0.01

… continued …

TABLE 1 Cont.

SGN-U214575	arabidopsis/peptide: At5g01650.1 68412.m00074 Macrophage migration inhibitory factor (MIF) family contains pfam profile: PF001187 Macrophage migration inhibitory factor (evalue: 1.1e-54, score=209.5) genbank/nr: gi|15241023|ref|NP_195785.1| Macrophage migration inhibitory factor (MIF) family [Arabidopsis thaliana] gi|11281345|pir||T48186 light-inducible protein ATLS1 - Arabidopsis thaliana gi|7327824|emb|CAB82281.1| light-inducible protein ATLS1 [Arabidopsis thaliana] gi|17065566|gb|AAL32937.1| light-inducible protein ATLS1 [Arabidopsis thaliana] gi|20148493|gb|AAM10137.1| light-inducible protein ATLS1 [Arabidopsis thaliana] (evalue: 3.7e-53, score=209.5)	1.73	0.07
SGN-U216352	arabidopsis/peptide: At2g45790.1 68409.m05148 phosphomannomutase -related (evalue: 4e-116, score=414.8) genbank/nr: gi|38345559|emb|CAE03433.2| OSJNBa0032F06.16 [Oryza sativa (japonica cultivar-group)] (evalue: 9e-119, score=428.7)	1.76	0.06
SGN-U217053	arabidopsis/peptide: At5g48370.1 68412.m05388 thioesterase family similar to SP|Q9R0X4 48 kDa acyl-CoA thioester hydrolase, mitochondrial precursor (EC 3.1.2.-) {Mus musculus}; contains Pfam profile PF03061: thioesterase family protein (evalue: 0, score=639.4) genbank/nr: gi|15238956|ref|NP_199648.1| thioesterase family [Arabidopsis thaliana] gi|8978341|dbj|BAA98194.1| contains similarity to acyl-CoA thioesterase~gene_id:K23F3.9 [Arabidopsis thaliana] (evalue: 0, score=639.4)	1.56	0.03
	METABOLISM		
SGN-U212562	arabidopsis/peptide: At5g17330.1 68412.m01869 glutamate decarboxylase 1 (GAD 1) sp|Q42521 (evalue: 1e-140, score=495.7) genbank/nr: gi|7436483|pir||T01962 glutamate decarboxylase (EC 4.1.1.15) 2, calmodulin-binding - common tobacco gi|3252854|gb|AAC39483.1| glutamate decarboxylase isozyme 2 [Nicotiana tabacum] (evalue: 3e-144, score=512.7)	1.79	0.05
SGN-U214499	arabidopsis/peptide: At4g32940.1 68411.m04261 gamma-VPE (vacuolar processing enzyme) (evalue: 0, score=714.5) genbank/nr: gi|27544012|dbj|BAC54830.1| vacuolar processing enzyme-3 [Nicotiana tabacum] (evalue: 0, score=866.7)	1.65	0.02
SGN-U216737	arabidopsis/peptide: At1g03150.1 68408.m00261 GCN5-related N-acetyltransferase (GNAT) family similar to SP|P07347 N-terminal acetyltransferase complex ARD1 subunit (Arrest-defective protein 1) {Saccharomyces cerevisiae}; contains Pfam profile PF00583: acetyltransferase, GNAT family (evalue: 1.3e-84, score=309.7) genbank/nr: gi|18379062|ref|NP_563677.1| GCN5-related N-acetyltransferase (GNAT) family [Arabidopsis thaliana] gi|21536510|gb|AAM60842.1| unknown [Arabidopsis thaliana] (evalue: 4.6e-83, score=309.7)	2.01	0.1

… continued …

TABLE 1 Cont.

SGN-U218165	arabidopsis/peptide: At1g32900.1 68408.m03672 starch synthase, putative similar to starch synthase SP:Q42857 from [Ipomoea batatas] (evalue: 2e-130, score=462.6) genbank/nr: gi|267196|sp|Q00775|SSG1_SOLTU Granule-bound starch synthase I, chloroplast precursor (GBSS I) >gi|66574|pir||YUPOY starch synthase (EC 2.4.1.21) precursor - potato >gi|21471|emb|CAA41359.1| glycogen (starch) synthase [Solanum tuberosum] (evalue: 2e-167, score=590.9)	1.74	0.04
	OTHERS		
AF320028	Lycopersicon esculentum heme oxygenase 1 (HO1) gene, complete cds; nuclear genefor chloroplast product	1.64	0.02
SGN-U213852	arabidopsis/peptide: At3g48740.1 68410.m04926 nodulin MtN3 family protein similar to MtN3 GI:1619602 (root nodule development) from [Medicago truncatula] (evalue: 3.4e-77, score=285) genbank/nr: gi|15229019|ref|NP_190443.1| nodulin MtN3 family protein [Arabidopsis thaliana] gi|11282600|pir||T46218 MTN3-like protein - Arabidopsis thaliana gi|6523105|emb|CAB62363.1| MTN3-like protein [Arabidopsis thaliana] gi|13605688|gb|AAK32837.1| AT3g48740/T8P19_250 [Arabidopsis thaliana] gi|16930411|gb|AAL31891.1| AT3g48740/T8P19_250 [Arabidopsis thaliana] gi|17979365|gb|AAL49908.1| putative MTN3 protein [Arabidopsis thaliana] gi|18700264|gb|AAL77742.1| AT3g48740/T8P19_250 [Arabidopsis thaliana] gi|20465523|gb|AAM20244.1| putative MTN3 protein [Arabidopsis thaliana] (evalue: 1.3e-75, score=285)	2.05	0.04
SGN-U213959	arabidopsis/peptide: At1g04580.1 68408.m00408 aldehyde oxidase, putative similar to aldehyde oxidases from Arabidopsis thaliana: GI:3172023, GI:3172025, GI:3172044 (evalue: 3e-89, score=325.1) genbank/nr: gi|10764218|gb|AAG22607.1| aldehyde oxidase [Lycopersicon esculentum] gi|14028575|gb|AAK52410.1| aldehyde oxidase TAO3 [Lycopersicon esculentum] (evalue: 1e-138, score=494.2)	1.45	0.06
SGN-U214814	arabidopsis/peptide: At2g06050.2 68415.m00664 12-oxophytodienoate reductase (OPR3) / delayed dehiscence1 (DDE1) nearly identical to DELAYED DEHISCENCE1 [GI:7688991] and to OPR3 [GI:10242314]; contains Pfam profile PF00724:oxidoreductase, FAD/FMN-binding; identical to cDNA OPDA-reductase homolog GI:5059114 (evalue: 3e-172, score=602.1) genbank/nr: gi|12056507|emb|CAC21424.1| 12-oxophytodienoate reductase 3 [Lycopersicon esculentum] (evalue: 0, score=801.2)	1.52	0.07

… continued …

TABLE 1 Cont.

SGN-U215281	arabidopsis/peptide: At2g36690.1 68409.m04074 oxidoreductase, 2OG-Fe(II) oxygenase family similar to IDS3 [Hordeum vulgare][GI:4514655], leucoanthocyanidin dioxygenase [SP|P51091][Malus domestica]; contains PF03171 2OG-Fe(II) oxygenase superfamily domain (evalue: 9e-63, score=237.7) genbank/nr: gi|42408583|dbj|BAD09760.1| putative flavonol synthase [Oryza sativa (japonica cultivar-group)] >gi|42409017|dbj|BAD10270.1| putative flavonol synthase [Oryza sativa (japonica cultivar-group)] (evalue: 9.3e-67, score=256.1)	1.53	0.06
SGN-U215649	arabidopsis/peptide: At2g03760.1 68409.m00299 steroid sulfotransferase, putative strong similarity to steroid sulfotransferases from [Brassica napus] GI:3420008, GI:3420004, GI:3420006; contains Pfam profile PF00685: Sulfotransferase domain (evalue: 3.3e-92, score=335.5) genbank/nr: gi|15227699|ref|NP_178471.1| steroid sulfotransferase, putative [Arabidopsis thaliana] gi|27735199|sp|P52839|FSTL_ARATH Flavonol sulfotransferase-like (RaRO47) gi|25288807|pir||A84452 probable steroid sulfotransferase [imported] - Arabidopsis thaliana gi|4406767|gb|AAD20078.1| putative steroid sulfotransferase [Arabidopsis thaliana] gi|14030735|gb|AAK53042.1| At2g03760/F19B11.21 [Arabidopsis thaliana] gi|21360485|gb|AAM47358.1| At2g03760/F19B11.21 [Arabidopsis thaliana] (evalue: 1.3e-90, score=335.5)	2.95	0.08
SGN-U215928	arabidopsis/peptide: At4g22220.1 68411.m02939 iron-sulfur cluster assembly complex protein, putative (ISCU1) similar to iron-sulfur cluster assembly complex ISCU1 (GI:11545705) [Homo sapiens]; nifU protein homolog YPL135w (GI:15619823) [Saccharomyces cerevisiae] PIR2:S69049 (evalue: 3.2e-65, score=245) genbank/nr: gi|34912076|ref|NP_917385.1| putative nifU-like protein [Oryza sativa (japonica cultivar-group)] gi|20521223|dbj|BAB91740.1| putative nifU-like protein [Oryza sativa (japonica cultivar-group)] (evalue: 1.6e-65, score=251.1)	1.74	0.07
SGN-U216025	arabidopsis/peptide: At5g19300.1 68412.m02105 hypothetical protein predicted proteins, H. sapiens, D. melanogaster and others (evalue: 2e-120, score=429.5) genbank/nr: gi|42567956|ref|NP_197431.2| expressed protein [Arabidopsis thaliana] >gi|45825145|gb|AAS77480.1| At5g19300 [Arabidopsis thaliana] (evalue: 8e-119, score=429.5)	1.7	0.01
SGN-U216398	arabidopsis/peptide: At4g28730.1 68411.m03744 glutaredoxin protein family contains glutaredoxin domain, Pfam:PF00462 (evalue: 2.9e-46, score=182.2) genbank/nr: gi|30688093|ref|NP_194602.2| glutaredoxin protein family [Arabidopsis thaliana] gi|26452363|dbj|BAC43267.1| unknown protein [Arabidopsis thaliana] gi|28372898|gb|AAO39931.1| At4g28730 [Arabidopsis thaliana] (evalue: 1.1e-44, score=182.2)	1.73	0.02

… continued …

TABLE 1 Cont.

SGN-U217201	arabidopsis/peptide: At4g25630.1 68411.m03360 fibrillarin 2 (AtFib2) identical to fibrillarin 2 GI:9965655 from [Arabidopsis thaliana] (evalue: 2e-124, score=441.8) genbank/nr: gi|18416588|ref|NP_567724.1| fibrillarin 2 (AtFib2) [Arabidopsis thaliana] gi|7450791|pir||T09555 fibrillarin - Arabidopsis thaliana gi|4914455|emb|CAB43694.1| fibrillarin-like protein [Arabidopsis thaliana] gi|7269413|emb|CAB81373.1| fibrillarin-like protein [Arabidopsis thaliana] gi|9965655|gb|AAG10104.1| fibrillarin 2 [Arabidopsis thaliana] gi|9965796|gb|AAG10153.1| fibrillarin 2 [Arabidopsis thaliana] gi|21536840|gb|AAM61172.1| fibrillarin 2 (AtFib2) [Arabidopsis thaliana] gi|23297150|gb|AAN13105.1| fibrillarin 2 (AtFib2) [Arabidopsis thaliana] (evalue: 7e-123, score=441.8)	1.71	0.1
SGN-U217291	arabidopsis/peptide: At5g14520.1 68412.m01569 pescadillo - like protein embryonic development allele: hi2, Danio rerio, EMBL:U77627pescadillo, Homo sapiens, EMBL:U78310 (evalue: 3e-160, score=562) genbank/nr: gi|28273368|gb|AAO38454.1| pescadillo-like protein [Oryza sativa (japonica cultivar-group)] (evalue: 8e-162, score=572.4)	1.53	0.08
SGN-U218491	arabidopsis/peptide: At2g31740.1 68409.m03514 expressed protein (evalue: 1.9e-61, score=233.4) genbank/nr: gi|34148076|gb|AAQ62585.1| putative spermine/spermidine synthase [Glycine max] (evalue: 2.6e-73, score=278.1)	1.59	0.07
SGN-U219582	arabidopsis/peptide: At4g37470.1 68411.m04822 hydrolase, alpha/beta fold family low similarity to SP|Q59093 3-oxoadipate enol-lactonase I (EC 3.1.1.24) (Enol-lactone hydrolase I) (Beta-ketoadipate enol-lactone hydrolase I) {Acinetobacter calcoaceticus}; contains Pfam profile PF00561: hydrolase, alpha/beta fold family (evalue: 2e-124, score=442.2) genbank/nr: gi|15235567|ref|NP_195463.1| hydrolase, alpha/beta fold family [Arabidopsis thaliana] gi|7450663|pir||T04741 hypothetical protein F6G17.120 - Arabidopsis thaliana gi|4468813|emb|CAB38214.1| putative protein [Arabidopsis thaliana] gi|7270729|emb|CAB80412.1| putative protein [Arabidopsis thaliana] gi|15810303|gb|AAL07039.1| unknown protein [Arabidopsis thaliana] gi|20259141|gb|AAM14286.1| unknown protein [Arabidopsis thaliana] (evalue: 8e-123, score=442.2)	1.57	0.06
SGN-U221073	arabidopsis/peptide: At5g42970.1 68412.m04727 COP9 complex subunit, FUS4 FUSCA4, COP8, CSN4; identical to COP8 GI:5802627 from [Arabidopsis thaliana] (evalue: 1.7e-74, score=275.4) genbank/nr: gi|33324486|gb|AAQ07984.1| COP8-like protein [Lilium longiflorum] (evalue: 2.4e-74, score=280)	1.64	0.06
SGN-U221084	arabidopsis/peptide: At1g60680.1 68408.m06265 aldo/keto reductase family contains Pfam profile PF00248: oxidoreductase, aldo/keto reductase family (evalue: 1.4e-51, score=199.5) genbank/nr: gi|37534402|ref|NP_921503.1| putative polyprotein [Oryza sativa (japonica cultivar-group)] gi|31432120|gb|AAP53790.1| putative polyprotein [Oryza sativa (japonica cultivar-group)] (evalue: 1.5e-62, score=241.1)	2.05	0.09

… continued …

TABLE 1 Cont.

SGN-U221863	arabidopsis/peptide: At3g50210.1 68410.m05087 oxidoreductase (din11), putative strong similarity to partial cds of 2-oxoacid-dependent oxidase (din11) from GI:10834554 [Arabidopsis thaliana] (evalue: 1.2e-63, score=239.2) genbank/nr: gi|6984228|gb|AAF34802.1| putative flavonol synthase-like protein [Euphorbia esula] (evalue: 8.8e-68, score=258.1)	1.72	0.06
SGN-U221959	arabidopsis/peptide: At2g47880.1 68409.m05397 glutaredoxin protein family contains INTERPRO Domain IPR002109, Glutaredoxin (thioltransferase) (evalue: 2.9e-36, score=149.4) genbank/nr: gi|15227151|ref|NP_182309.1| glutaredoxin protein family [Arabidopsis thaliana] gi|25282784|pir||F84920 probable glutaredoxin [imported] - Arabidopsis thaliana gi|3738300|gb|AAC63642.1| putative glutaredoxin [Arabidopsis thaliana] gi|20197557|gb|AAM15127.1| putative glutaredoxin [Arabidopsis thaliana] gi|21554200|gb|AAM63279.1| putative glutaredoxin [Arabidopsis thaliana] (evalue: 1.1e-34, score=149.4)	2.11	0.06
SGN-U223643	arabidopsis/peptide: At2g42490.1 68409.m04750 copper amine oxidase -related (evalue: 2e-170, score=594.7) genbank/nr: gi|5230728|gb|AAD40979.1| peroxisomal copper-containing amine oxidase [Glycine max] (evalue: 7e-175, score=615.1)	2.02	0.05
SGN-U223935	arabidopsis/peptide: At4g08790.1 68411.m01305 nitrilase 1 like protein nitrilase 1 - Mus musculus,PID:g3228668 (evalue: 9e-123, score=436.8) genbank/nr: gi|18413157|ref|NP_567340.1| nitrilase 1 like protein [Arabidopsis thaliana] gi|13926307|gb|AAK49620.1| AT4g08790/T32A17_100 [Arabidopsis thaliana] gi|22137058|gb|AAM91374.1| At4g08790/T32A17_100 [Arabidopsis thaliana] (evalue: 3e-121, score=436.8)	1.54	0.06
SGN-U225236	arabidopsis/peptide: At3g26040.1 68410.m02970 acyltransferase family similar to deacetylvindoline 4-O-acetyltransferase [Catharanthus roseus][GI:4091808][PMID:9681034], alcohol acyltransferase [Fragaria x ananassa][GI:10121328][PMID:10810141] (evalue: 1.2e-29, score=127.1) genbank/nr: gi|48210045|gb|AAT40544.1| putative acetyltranferase [Solanum demissum] (evalue: 3e-29, score=131)	1.7	0.09
	OXIDATIVE BURST / HYPERSENSITIVE RESPONSE		
SGN-U212914	arabidopsis/peptide: At1g08830.1 68408.m00884 copper/zinc superoxidase dismutase (CSD1) identical to SWISS-PROT: P24704 (evalue: 4.6e-70, score=261.2) genbank/nr: gi|3334337|sp|Q43779|SOD2_LYCES Superoxide dismutase [Cu-Zn] 2 gi|1084402|pir||S55402 superoxide dismutase (EC 1.15.1.1) (Cu-Zn), cytosolic - tomato gi|854248|emb|CAA60826.1| cytosolic Cu,Zn superoxide dismutase [Lycopersicon esculentum] (evalue: 1.2e-79, score=298.1)	1.63	0.1
SGN-U213351	arabidopsis/peptide: At1g71695.1 68408.m07598 peroxidase, putative identical to GB:CAA67309 GI:1429213 from [Arabidopsis thaliana] (evalue: 1.1e-83, score=307) genbank/nr: gi|14031049|gb|AAK52084.1| peroxidase [Nicotiana tabacum] (evalue: 4.7e-91, score=336.7)	2.11	0.11

… continued …

TABLE 1 Cont.

SGN-U214733	arabidopsis/peptide: At4g11600.1 68411.m01682 glutathione peroxidase, putative (evalue: 8.2e-79, score=290.4) genbank/nr: gi|20138152|sp|O24031|GPX4_LYCES Probable phospholipid hydroperoxide glutathione peroxidase (PHGPx) gi|2388885|emb|CAA75054.1| glutathione peroxidase [Lycopersicon esculentum] (evalue: 1.8e-93, score=344.4)	1.61	0.06
SGN-U214812	arabidopsis/peptide: At4g31870.1 68411.m04123 glutathione peroxidase, putative glutathione peroxidase, Arabidopsis thaliana, PIR2:S71250 (evalue: 5.9e-86, score=314.3) genbank/nr: gi|20138099|sp|O24296|GPX1_PEA Phospholipid hydroperoxide glutathione peroxidase, chloroplast precursor (PHGPx) gi|7433115|pir||T06462 glutathione peroxidase (EC 1.11.1.9) precursor - garden pea gi|2632109|emb|CAA04142.1| phospholipid glutathione peroxidase [Pisum sativum] (evalue: 7.6e-85, score=315.8)	1.41	0.07
SGN-U216820	arabidopsis/peptide: At1g63460.1 68408.m06583 glutathione peroxidase, putative contains Pfam profile: PF00255 glutathione peroxidases (evalue: 4.7e-71, score=264.2) genbank/nr: gi|18407822|ref|NP_564813.1| glutathione peroxidase, putative [Arabidopsis thaliana] gi|21592603|gb|AAM64552.1| unknown [Arabidopsis thaliana] gi|27765006|gb|AAO23624.1| At1g63460 [Arabidopsis thaliana] (evalue: 1.7e-69, score=264.2)	1.52	0.31
SGN-U219084	arabidopsis/peptide: At2g48150.1 68409.m05437 glutathione peroxidase, putative (evalue: 7e-69, score=257.3) genbank/nr: gi|18407538|ref|NP_566128.1| glutathione peroxidase, putative [Arabidopsis thaliana] gi|21617962|gb|AAM67012.1| putative glutathione peroxidase [Arabidopsis thaliana] gi|26451929|dbj|BAC43057.1| putative glutathione peroxidase [Arabidopsis thaliana] gi|28372962|gb|AAO39963.1| At2g48150 [Arabidopsis thaliana] (evalue: 2.6e-67, score=257.3)	1.6	0.01
SGN-U219399	arabidopsis/peptide: At1g44970.1 68408.m04722 peroxidase, putative similar to peroxidase GI:993004 from [Mercurialis annua] (evalue: 3e-137, score=485) genbank/nr: gi|14031051|gb|AAK52085.1| peroxidase [Nicotiana tabacum] (evalue: 9e-155, score=548.5)	1.62	0.06
SGN-U212749	arabidopsis/peptide: At3g09270.1 68410.m00987 glutathione transferase, putative similar to glutathione transferase GB:CAA71784 [Glycine max] (evalue: 4.6e-51, score=197.6) genbank/nr: gi|416649|sp|Q03662|GTX1_TOBAC Probable glutathione S-transferase (Auxin-induced protein PGNT1/PCNT110) gi|100303|pir||S16267 auxin-induced protein (clones pGNT1 and pCNT110) - common tobacco gi|19789|emb|CAA39709.1| auxin-induced protein [Nicotiana tabacum] gi|19795|emb|CAA39705.1| auxin-induced protein [Nicotiana tabacum] (evalue: 1.1e-82, score=307.8)	2.6	0.03
SGN-U215385	arabidopsis/peptide: At1g71695.1 68408.m07598 peroxidase, putative identical to GB:CAA67309 GI:1429213 from [Arabidopsis thaliana] (evalue: 3e-116, score=415.2) genbank/nr: gi|14031049|gb|AAK52084.1| peroxidase [Nicotiana tabacum] (evalue:	1.67	0.01

… continued …

TABLE 1 Cont.

	3e-166, score=587)		
SGN-U215794	arabidopsis/peptide: At2g28190.1 68409.m03098 copper/zinc superoxide dismutase (CSD2) identical to GP:3273753:AF061519 (evalue: 4.3e-67, score=251.5) genbank/nr: gi|33327349|gb|AAQ09007.1| superoxidase dismutase [Lycopersicon esculentum] (evalue: 2.7e-81, score=303.9)	1.44	0.1
SGN-U216874	arabidopsis/peptide: At1g06130.2 68408.m08983 glyoxalase II, putative (hydroxyacylglutathione hydrolase) similar to glyoxalase II isozyme GB:AAC49865 GI:2570338 from [Arabidopsis thaliana] (evalue: 1e-124, score=443.7) genbank/nr: gi|30679573|ref|NP_849599.1| glyoxalase II, putative (hydroxyacylglutathione hydrolase) [Arabidopsis thaliana] gi|20466237|gb|AAM20436.1| glyoxalase II isozyme, putative [Arabidopsis thaliana] gi|22136310|gb|AAM91233.1| glyoxalase II isozyme, putative [Arabidopsis thaliana] (evalue: 4e-123, score=443.7)	1.62	0.05
SGN-U216884	arabidopsis/peptide: At5g41210.1 68412.m04513 glutathione transferase, putative similar to emb|CAA10662 (evalue: 1.3e-92, score=336.7) genbank/nr: gi|11385461|gb|AAG34813.1| glutathione S-transferase GST 23 [Glycine max] (evalue: 5.2e-96, score=353.2)	2.18	0.01
SGN-U225169	arabidopsis/peptide: At5g04660.1 68412.m00439 cytochrome P450, putative cytochrome P450 77A3p, Glycine max., PIR:T05948 (evalue: 1e-62, score=236.1) genbank/nr: gi|584867|sp|P37124|C772_SOLME Cytochrome P450 77A2 (CYPLXXVIIA2) (P-450EG5) gi|542071|pir||S41598 cytochrome P450 77A2 - eggplant gi|438241|emb|CAA50646.1| CYP77A2 [Solanum melongena] (evalue: 3.3e-83, score=309.3)	1.91	0.04
	PHOTOSYNTHESIS		
SGN-U215807	arabidopsis/peptide: At1g01300.1 68408.m00037 chloroplast nucleoid DNA binding protein -related similar to chloroplast nucleoid DNA binding protein GB:BAA22813 GI:2541876 from [Nicotiana tabacum] (evalue: 2e-158, score=556.2) genbank/nr: gi|15223368|ref|NP_171637.1| chloroplast nucleoid DNA binding protein -related [Arabidopsis thaliana] gi|25518405|pir||C86143 hypothetical protein F6F3.10 - Arabidopsis thaliana gi|9665144|gb|AAF97328.1| Unknown protein [Arabidopsis thaliana] gi|22135930|gb|AAM91547.1| chloroplast nucleoid DNA binding protein, putative [Arabidopsis thaliana] gi|30387595|gb|AAP31963.1| At1g01300 [Arabidopsis thaliana] (evalue: 6e-157, score=556.2)	1.59	0
SGN-U215851	arabidopsis/peptide: At3g15360.1 68410.m01749 thioredoxin M-type 4, chloroplast precursor (TRX-M4) nearly identical to SP|Q9SEU6 Thioredoxin M-type 4, chloroplast precursor (TRX-M4) {Arabidopsis thaliana} (evalue: 2.2e-46, score=182.6) genbank/nr: gi|15594012|emb|CAC69854.1| putative thioredoxin m2 [Pisum sativum] (evalue: 1.9e-54, score=214.5)	1.77	0.05

… continued …

TABLE 1 Cont.

SGN-U218193	arabidopsis/peptide: At5g27380.1 68412.m02976 glutathione synthetase (GSH2) non-consensus AT donor splice site at exon 6, AC acceptor splice site at exon 7 (evalue: 0, score=686.8) genbank/nr: gi|20138145|sp|O22494|GSHB_LYCES Glutathione synthetase, chloroplast precursor (Glutathione synthase) (GSH synthetase) (GSH-S) gi|7489006|pir||T04336 glutathione synthase (EC 6.3.2.3) 2 - tomato gi|2407617|gb|AAB71231.1| glutathione synthetase [Lycopersicon esculentum] (evalue: 0, score=1070.5)	1.48	0.1
	PROTEIN BIOSYNTHESIS		
SGN-U212746	arabidopsis/peptide: At2g16600.1 68409.m01716 cytosolic cyclophilin (ROC3) (evalue: 6.6e-74, score=273.9) genbank/nr: gi|118103|sp|P21568|CYPH_LYCES Peptidyl-prolyl cis-trans isomerase (PPIase) (Rotamase) (Cyclophilin) (Cyclosporin A-binding protein) gi|170440|gb|AAA63543.1| cyclophilin (evalue: 1.4e-88, score=327.8)	1.59	0.03
SGN-U212967	arabidopsis/peptide: At2g27530.2 68409.m05661 60S ribosomal protein L10A (RPL10aB) (evalue: 7.9e-97, score=350.5) genbank/nr: gi|18401451|ref|NP_565654.1| 60S ribosomal protein L10A (RPL10aB) [Arabidopsis thaliana] gi|30683566|ref|NP_850104.1| 60S ribosomal protein L10A (RPL10aB) [Arabidopsis thaliana] gi|27923989|sp|P59230|R10B_ARATH 60S ribosomal protein L10a-2 gi|13430468|gb|AAK25856.1| putative 60S ribosomal protein L10A [Arabidopsis thaliana] gi|15810665|gb|AAL07257.1| putative 60S ribosomal protein L10A [Arabidopsis thaliana] gi|19698833|gb|AAL91152.1| 60S ribosomal protein L10A [Arabidopsis thaliana] gi|20197452|gb|AAC73045.2| 60S ribosomal protein L10A [Arabidopsis thaliana] gi|20197665|gb|AAM15190.1| 60S ribosomal protein L10A [Arabidopsis thaliana] gi|30023674|gb|AAP13370.1| At2g27530 [Arabidopsis thaliana] (evalue: 3e-95, score=350.5)	1.56	0.05
SGN-U213207	arabidopsis/peptide: At3g09200.1 68410.m00980 60S acidic ribosomal protein P0 (RPP0B) similar to putative 60S acidic ribosomal protein P0 GB:P50346 [Glycine max] (evalue: 1e-124, score=443.4) genbank/nr: gi|1710587|sp|P50346|RLA0_SOYBN 60S ACIDIC RIBOSOMAL PROTEIN P0 gi|7440740|pir||T07106 acidic ribosomal protein P0 - soybean gi|1196897|gb|AAB63814.1| acidic ribosomal protein P0 [Glycine max] (evalue: 1e-125, score=451.8)	1.76	0.1
SGN-U213445	arabidopsis/peptide: At3g11510.1 68410.m01267 40S ribosomal protein S14 (RPS14B) similar to 40S ribosomal protein S14 GB:P19950 [Zea mays] (evalue: 8.4e-60, score=226.9) genbank/nr: gi|28436071|gb|AAO41731.1| cytoplasmic ribosomal protein S14 [Brassica napus] (evalue: 1.2e-59, score=231.5)	1.41	0.01

… continued …

TABLE 1 Cont.

SGN-U213502	arabidopsis/peptide: At3g13920.1 68410.m01577 eukaryotic translation initiation factor 4A-1 (eIF4A-1) eIF-4A-1 gi:15293046, gi:15450485 (evalue: 0, score=771.2) genbank/nr: gi|1170506|sp|P41379|IF42_NICPL Eukaryotic initiation factor 4A-2 (eIF4A-2) (eIF-4A-2) gi|100275|pir||S22578 translation initiation factor eIF-4A - curled-leaved tobacco gi|19697|emb|CAA43513.1| nicotiana eukaryotic translation initiation factor 4A [Nicotiana plumbaginifolia] (evalue: 0, score=811.6)	1.82	0
SGN-U215228	arabidopsis/peptide: At4g16520.2 68411.m05465 symbiosis-related like protein (evalue: 6.5e-57, score=217.2) genbank/nr: gi|21615419|emb|CAD33929.1| microtubule associated protein [Cicer arietinum] (evalue: 1.4e-57, score=224.6)	1.43	0.02
SGN-U215438	arabidopsis/peptide: At5g58710.1 68412.m06653 cyclophilin ROC7 (evalue: 1.7e-87, score=319.3) genbank/nr: gi|15237739|ref|NP_200679.1| cyclophilin ROC7 [Arabidopsis thaliana] gi|11270328|pir||T50838 peptidylprolyl isomerase (EC 5.2.1.8) ROC7 [similarity] - Arabidopsis thaliana gi|6180043|gb|AAF05760.1| cyclophilin [Arabidopsis thaliana] gi|8843791|dbj|BAA97339.1| cyclophilin [Arabidopsis thaliana] gi|15081670|gb|AAK82490.1| AT5g58710/mzn1_160 [Arabidopsis thaliana] gi|20334834|gb|AAM16173.1| AT5g58710/mzn1_160 [Arabidopsis thaliana] gi|21554366|gb|AAM63473.1| cyclophilin ROC7 [Arabidopsis thaliana] (evalue: 6.3e-86, score=319.3)	1.61	0.09
SGN-U217073	arabidopsis/peptide: At3g06680.1 68410.m00718 60S ribosomal protein L29 (RPL29B) similar to 60S ribosomal protein L29 GB:P25886 from (Rattus norvegicus) (evalue: 1.3e-23, score=106.3) genbank/nr: gi|12231298|gb|AAG49033.1| ripening regulated protein DDTFR19 [Lycopersicon esculentum] (evalue: 9.1e-25, score=115.2)	1.62	0.05
SGN-U219046	arabidopsis/peptide: At3g57080.1 68410.m05871 eukaryotic rpb5 RNA polymerase subunit family similar to SP|P19388 DNA-directed RNA polymerase II 23 kDa polypeptide (EC 2.7.7.6) {Homo sapiens}; contains Pfam profiles PF03871: RNA polymerase Rpb5 N-terminal domain, PF01191: RNA polymerase Rpb5 C-terminal domain (evalue: 1.9e-65, score=246.1) genbank/nr: gi|15230206|ref|NP_191267.1| eukaryotic rpb5 RNA polymerase subunit family [Arabidopsis thaliana] gi|11261045|pir||T47768 hypothetical protein F24I3.160 - Arabidopsis thaliana gi|6911878|emb|CAB72178.1| putative protein [Arabidopsis thaliana] gi|26452919|dbj|BAC43537.1| unknown protein [Arabidopsis thaliana] gi|28973011|gb|AAO63830.1| unknown protein [Arabidopsis thaliana] (evalue: 7.1e-64, score=246.1)	1.7	0.03
SGN-U223212	arabidopsis/peptide: At3g07880.1 68410.m00870 RHO GDP-dissociation inhibitor 1 -related similar to RHO GDP-dissociation inhibitor 1 GB:P19803 [Bos taurus] (evalue: 1.3e-47, score=187.2) genbank/nr: gi|7228160|emb|CAB77025.1| putative Rho GDP dissociation inhibitor [Nicotiana tabacum] (evalue: 1.5e-95, score=351.7)	1.56	0.01

… continued …

TABLE 1 Cont.

SGN-U225241	arabidopsis/peptide: At5g05920.1 68412.m00608 deoxyhypusine synthase (evalue: 1e-138, score=489.6) genbank/nr: gi|38503163|sp|Q9AXR0|DHYS_LYCES Deoxyhypusine synthase >gi|12407775|gb|AAG53641.1| deoxyhypusine synthase [Lycopersicon esculentum] (evalue: 3e-173, score=610.1)	1.71	0.03
	PROTEIN DEGRADATION		
SGN-U212891	arabidopsis/peptide: At2g02760.1 68409.m00192 ubiquitin-conjugating enzyme 2 (UBC2) E2; identical to gi:2689242, SP:P42745 (evalue: 1.8e-86, score=315.8) genbank/nr: gi|8118527|gb|AAF73016.1| ubiquitin conjugating protein [Avicennia marina] (evalue: 1.4e-85, score=318.2)	1.53	0.1
SGN-U213149	arabidopsis/peptide: At5g53300.2 68412.m07968 ubiquitin-conjugating enzyme 10 (UBC10) E2; identical to gi:297877, SP:P35133 (evalue: 1.3e-83, score=306.2) genbank/nr: gi|5762457|gb|AAD51109.1| ubiquitin-conjugating enzyme UBC2 [Mesembryanthemum crystallinum] (evalue: 9.7e-83, score=308.5)	1.64	0.03
SGN-U213613	arabidopsis/peptide: At5g43580.1 68412.m04803 hypothetical protein (evalue: 3.6e-08, score=54.3) genbank/nr: gi|124192|sp|P20076|IER1_LYCES Ethylene-responsive proteinase inhibitor I precursor gi|82085|pir||A32067 ethylene-responsive proproteinase inhibitor I precursor - tomato gi|623594|gb|AAA60745.1| proteinase inhibitor I (evalue: 1e-09, score=64.31)	2.36	0.04
SGN-U214642	arabidopsis/peptide: At1g45000.1 68408.m04725 26S proteasome regulatory particle triple-A ATPase subunit4 -related similar to 26S proteasome regulatory particle triple-A ATPase subunit4 GI:11094192 from [Oryza sativa] (evalue: 0, score=736.1) genbank/nr: gi|24745880|dbj|BAC23035.1| 26S proteasome AAA-ATPase subunit RPT4a [Solanum tuberosum] (evalue: 0, score=777.3)	2.09	0.01
SGN-U217107	arabidopsis/peptide: At3g45010.1 68410.m04491 serine carboxypeptidase III, putative similar to serine carboxypeptidase III from Oryza sativa SP|P37891, Matricaria chamomilla GI:6960455, Hordeum vulgare SP|P21529, Triticum aestivum SP|P11515; contains Pfam profile PF0450 serine carboxypeptidase (evalue: 1.4e-99, score=359.4) genbank/nr: gi|6960455|gb|AAD42963.2| serine carboxypeptidase precursor [Matricaria chamomilla] (evalue: 5e-101, score=369.4)	1.73	0.09
SGN-U219136	arabidopsis/peptide: At4g24690.1 68411.m03221 ubiquitin-associated (UBA)/PB1 domain-containing protein contains Pfam profiles PF00627: Ubiquitin-associated (UBA)/TS-N domain, PF00569: Zinc finger ZZ type domain, PF00564: PB1 domain (evalue: 2.6e-80, score=295.8) genbank/nr: gi|25044803|gb|AAM28274.1| PFE18 protein [Ananas comosus] (evalue: 7.6e-79, score=296.2)	1.55	0.03
SGN-U219551	arabidopsis/peptide: At5g42220.1 68412.m04634 ubiquitin family contains INTERPRO:IPR000626 ubiquitin domain (evalue: 1e-70, score=264.6) genbank/nr: gi|10177007|dbj|BAB10195.1| gene_id:K5J14.2~pir||T30561~similar to unknown protein [Arabidopsis thaliana] (evalue: 3.9e-69, score=264.6)	1.71	0.09

… continued …

TABLE 1 Cont.

SGN-U220585	arabidopsis/peptide: At1g22050.1 68408.m02495 ubiquitin family contains INTERPRO:IPR000626 ubiquitin domain (evalue: 1.2e-37, score=153.7) genbank/nr: gi|4097587|gb|AAD00119.1| NTGP5 [Nicotiana tabacum] (evalue: 4.5e-49, score=196.8)	2.03	0.08
SGN-U220965	arabidopsis/peptide: At3g07990.1 68410.m00881 serine carboxypeptidase -related similar to serine carboxypeptidase II (CP-MII) GB:CAA70815 [Hordeum vulgare] (evalue: 5.5e-82, score=300.8) genbank/nr: gi|15231911|ref|NP_187456.1| serine carboxypeptidase -related [Arabidopsis thaliana] gi|6648211|gb|AAF21209.1| putative serine carboxypeptidase II [Arabidopsis thaliana] (evalue: 2e-80, score=300.8)	1.65	0.03
	SIGNAL TRANSDUCTION		
SGN-U212665	arabidopsis/peptide: At3g51850.1 68410.m05270 calcium-dependent protein kinase, putative (CDPK) similar to calcium-dependent protein kinase [Arabidopsis thaliana] gi|836942|gb|AAA67655; contains protein kinase domain, Pfam:PF00069; contains EF hand domain (calcium-binding EF-hand), Pfam:PF00036, INTERPRO:IPR002048 (evalue: 1e-162, score=571.2) genbank/nr: gi|17064926|gb|AAL32617.1| calcium-dependent protein kinase [Arabidopsis thaliana] gi|28059078|gb|AAO29985.1| calcium-dependent protein kinase [Arabidopsis thaliana] (evalue: 4e-161, score=571.2)	1.49	0.09
SGN-U212665	arabidopsis/peptide: At3g51850.1 68410.m05270 calcium-dependent protein kinase, putative (CDPK) similar to calcium-dependent protein kinase [Arabidopsis thaliana] gi|836942|gb|AAA67655; contains protein kinase domain, Pfam:PF00069; contains EF hand domain (calcium-binding EF-hand), Pfam:PF00036, INTERPRO:IPR002048 (evalue: 1e-162, score=571.2) genbank/nr: gi|17064926|gb|AAL32617.1| calcium-dependent protein kinase [Arabidopsis thaliana] gi|28059078|gb|AAO29985.1| calcium-dependent protein kinase [Arabidopsis thaliana] (evalue: 4e-161, score=571.2)	1.55	0.06
SGN-U212854	arabidopsis/peptide: At3g43810.1 68410.m04339 calmodulin almost identical to calmodulin GI:16227 from [Arabidopsis thaliana] (evalue: 6.3e-66, score=248.8) genbank/nr: gi|115525|sp|P04353|CALM_SPIOL Calmodulin gi|71685|pir||MCSP calmodulin - spinach (tentative sequence) (evalue: 2.5e-64, score=248.8)	2.42	0.01
SGN-U213545	arabidopsis/peptide: At3g43810.1 68410.m04339 calmodulin almost identical to calmodulin GI:16227 from [Arabidopsis thaliana] (evalue: 2.1e-76, score=282) genbank/nr: gi|115513|sp|P27161|CALM_LYCES Calmodulin gi|170396|gb|AAA34144.1| calmodulin gi|3549695|emb|CAA09302.1| calmodulin 3 protein [Capsicum annuum] gi|14625401|dbj|BAB61907.1| calmodulin NtCaM1 [Nicotiana tabacum] gi|14625403|dbj|BAB61908.1| calmodulin NtCaM2 [Nicotiana tabacum] gi|21616059|emb|CAC84563.1| putative calmodulin [Solanum commersonii] (evalue: 4.9e-79, score=295.8)	1.53	0.01

… continued …

TABLE 1 Cont.

SGN-U214843	arabidopsis/peptide: At4g19110.2 68411.m05258 protein kinase, putative contains protein kinase domain, Pfam:PF00069 (evalue: 5e-171, score=598.6) genbank/nr: gi|30684655|ref|NP_849407.1| protein kinase, putative [Arabidopsis thaliana] (evalue: 2e-169, score=598.6)	1.53	0.1
SGN-U215082	arabidopsis/peptide: At1g73500.1 68408.m07808 mitogen-activated protein kinase kinase (MAPKK), putative (MKK9) mitogen-activated protein kinase kinase (MAPKK) family, PMID:12119167 (evalue: 2e-101, score=366.3) genbank/nr: gi|15219482|ref|NP_177492.1| mitogen-activated protein kinase kinase (MAPKK), putative (MKK9) [Arabidopsis thaliana] gi|25287619|pir||G96761 probable MAP kinase T9L24.32 [imported] - Arabidopsis thaliana gi|11120804|gb|AAG30984.1| MAP kinase, putative [Arabidopsis thaliana] gi|21536805|gb|AAM61137.1| MAP kinase, putative [Arabidopsis thaliana] gi|26452087|dbj|BAC43133.1| unknown protein [Arabidopsis thaliana] gi|28950881|gb|AAO63364.1| At1g73500 [Arabidopsis thaliana] (evalue: 7e-100, score=366.3)	2.05	0.01
SGN-U215107	arabidopsis/peptide: At1g21410.1 68408.m02420 F-box protein family similar to SKP1 interacting partner 2 (SKIP2) TIGR_Ath1:At5g67250 (evalue: 1.2e-52, score=117.1) genbank/nr: gi|21554029|gb|AAM63110.1| F-box protein AtFBL5 [Arabidopsis thaliana] (evalue: 3.8e-51, score=117.1)	1.56	0.03
SGN-U216024	arabidopsis/peptide: At5g48380.1 68412.m05390 leucine rich repeat protein family contains protein kinase domain, Pfam:PF00069; contains leucine-rich repeats, Pfam:PF00560 (evalue: 0, score=716.8) genbank/nr: gi|18422906|ref|NP_568696.1| leucine rich repeat protein family [Arabidopsis thaliana] gi|13605827|gb|AAK32899.1| AT5g48380/MJE7_1 [Arabidopsis thaliana] gi|18389278|gb|AAL67082.1| putative receptor protein kinase [Arabidopsis thaliana] (evalue: 0, score=716.8)	1.87	0.1
SGN-U216378	arabidopsis/peptide: At3g52180.1 68410.m05308 expressed protein (evalue: 4e-134, score=474.9) genbank/nr: gi|14970762|emb|CAC44460.1| protein tyrosine phosphatase [Lycopersicon esculentum] (evalue: 0, score=754.2)	1.52	0.1
SGN-U216696	arabidopsis/peptide: At4g28400.1 68411.m03704 protein phosphatase 2C (PP2C), putative protein phosphatase 2C-fission yeast, PIR2:S54297 (evalue: 3e-103, score=372.1) genbank/nr: gi|46277128|gb|AAS86762.1| protein phosphatase 2C [Lycopersicon esculentum] (evalue: 9e-103, score=375.9)	1.65	0.02
SGN-U216698	arabidopsis/peptide: At1g13900.1 68408.m01475 calcineurin-like phosphoesterase family contains Pfam profile: PF00149 calcineurin-like phosphoesterase (evalue: 6e-101, score=364.8) genbank/nr: gi|15222978|ref|NP_172843.1| calcineurin-like phosphoesterase family [Arabidopsis thaliana] gi|8778406|gb|AAF79414.1| F16A14.11 [Arabidopsis thaliana] (evalue: 2.2e-99, score=364.8)	1.8	0.05

… continued …

TABLE 1 Cont.

SGN-U220589	arabidopsis/peptide: At3g11410.1 68410.m01257 protein phosphatase 2C (PP2C), putative identical to protein phosphatase 2C (PP2C) GB:P49598 [Arabidopsis thaliana] (evalue: 5e-47, score=184.5) genbank/nr: gi|4336434|gb|AAD17804.1| nodule-enhanced protein phosphatase type 2C [Lotus japonicus] (evalue: 8.4e-51, score=202.2)	1.95	0.04
SGN-U222721	arabidopsis/peptide: At4g28600.1 68411.m03727 calmodulin-binding protein similar to pollen-specific calmodulin-binding protein MPCBP GI:10086260 from [Zea mays] (evalue: 4.5e-84, score=308.1) genbank/nr: gi|22329002|ref|NP_194589.2| calmodulin-binding protein [Arabidopsis thaliana] (evalue: 1.7e-82, score=308.1)	1.78	0.02
SGN-U223724	arabidopsis/peptide: At3g11410.1 68410.m01257 protein phosphatase 2C (PP2C), putative identical to protein phosphatase 2C (PP2C) GB:P49598 [Arabidopsis thaliana] (evalue: 7.5e-46, score=127.5) genbank/nr: gi|10432446|emb|CAC10358.1| protein phosphatase 2C [Nicotiana tabacum] gi|22553023|emb|CAC84141.2| protein phosphatase 2C [Nicotiana tabacum] (evalue: 1.3e-55, score=162.5)	1.46	0.02
SGN-U225762	arabidopsis/peptide: At5g43920.1 68412.m04843 transducin / WD-40 repeat protein family contains 7 WD-40 repeats (PF00400); similar to will die slowly protein (WDS) (SP:Q9V3J8) [Drosophila melanogaster] (evalue: 5.8e-72, score=267.3) genbank/nr: gi|15240036|ref|NP_199205.1| transducin / WD-40 repeat protein family [Arabidopsis thaliana] gi|9758551|dbj|BAB09052.1| WD-repeat protein-like [Arabidopsis thaliana] (evalue: 2.1e-70, score=267.3)	1.72	0.1
SGN-U226074	arabidopsis/peptide: At4g11655.1 68417.m01863 transmembrane protein, putative contains 4 transmembrane spanning domains, PMID:11152613 (evalue: 1e-28, score=124.4) genbank/nr: gi|42572877|ref|NP_974535.1| transmembrane protein, putative [Arabidopsis thaliana] (evalue: 4e-27, score=124.4)	1.96	0.03
SGN-U226415	arabidopsis/peptide: At1g53430.1 68408.m05556 receptor-related serine/threonine kinase similar to receptor-like serine/threonine kinase GB:AAC50043 GI:2465923 from [Arabidopsis thaliana] (evalue: 1.8e-56, score=215.7) genbank/nr: gi|38228683|emb|CAE54078.1| receptor-like protein kinase [Fagus sylvatica] (evalue: 8.6e-57, score=221.9)	1.97	0.02
SGN-U227357	arabidopsis/peptide: At1g71010.1 68408.m07513 expressed protein (evalue: 7.2e-55, score=124.4) genbank/nr: gi|42563125|ref|NP_177257.3| phosphatidylinositol-4-phosphate 5-kinase family protein [Arabidopsis thaliana] gi|5902400|gb|AAD55502.1| Unknown protein [Arabidopsis thaliana] (evalue: 2.7e-53, score=124.4)	2.23	0.02
SGN-U227773	arabidopsis/peptide: At1g49740.1 68408.m05109 expressed protein similar to MAP3K-like protein kinase GB:CAB16796 GI:4006878 from [Arabidopsis thaliana] (evalue: 1e-102, score=369.4) genbank/nr: gi|25407778|pir||D85436 MAP3K-like protein kinase [imported] - Arabidopsis thaliana gi|4006878|emb|CAB16796.1| MAP3K-like protein kinase [Arabidopsis thaliana] gi|7270644|emb|CAB80361.1| MAP3K-like protein kinase [Arabidopsis thaliana] (evalue: 7e-105, score=382.1)	1.52	0.02

… continued …

TABLE 1 Cont.

SGN-U230493	arabidopsis/peptide: At1g30440.1 68408.m03369 phototropic response protein family contains NPH3 family domain, Pfam:PF03000 (evalue: 4e-58, score=220.3) genbank/nr: gi|15220750|ref|NP_174332.1| phototropic response protein family [Arabidopsis thaliana] (evalue: 1.2e-56, score=220.3)	1.75	0.01
	STRESS RESPONSE		
SGN-U214993	arabidopsis/peptide: At5g02380.1 68412.m00149 metallothionein 2b (evalue: 4.3e-07, score=51.22) genbank/nr: gi|2497896|sp|Q40157|MT2A_LYCES Metallothionein-like protein type 2 A (LeMT(A)) gi|7441812|pir||T07073 metallothionein type II A - tomato gi|1449136|gb|AAB04674.1| metallothionein II-like protein [Lycopersicon esculentum] (evalue: 1.2e-23, score=111.3)	1.48	0.04
SGN-U216624	arabidopsis/peptide: At1g61770.1 68408.m06387 DnaJ protein family similar to SP|Q9UBS4 DnaJ homolog subfamily B member 11 precursor Homo sapiens; contains Pfam profile PF00226 DnaJ domain (evalue: 4e-118, score=421.8) genbank/nr: gi|30696610|ref|NP_176370.2| DnaJ protein family [Arabidopsis thaliana] gi|26983836|gb|AAN86170.1| unknown protein [Arabidopsis thaliana] (evalue: 1e-116, score=421.8)	1.46	0.04
SGN-U221325	arabidopsis/peptide: At4g28480.1 68411.m03712 heat shock protein, putative similar to SP|Q9UDY4|DJB4_HUMAN DnaJ homolog subfamily B member 4 (Heat shock 40 kDa protein 1 homolog) {Homo sapiens}; contains Pfam profile PF00226: DnaJ domain (evalue: 1.1e-58, score=223.8) genbank/nr: gi|15235310|ref|NP_194577.1| heat shock protein, putative [Arabidopsis thaliana] gi|7441938|pir||T04618 heat shock protein homolog F20O9.160 - Arabidopsis thaliana gi|2842490|emb|CAA16887.1| heat-shock protein [Arabidopsis thaliana] gi|7269702|emb|CAB79650.1| heat-shock protein [Arabidopsis thaliana] gi|14596115|gb|AAK68785.1| heat-shock protein [Arabidopsis thaliana] gi|20148389|gb|AAM10085.1| heat-shock protein [Arabidopsis thaliana] (evalue: 4e-57, score=223.8)	1.84	0.04
SGN-U221384	arabidopsis/peptide: At1g71950.1 68408.m07632 expressed protein similar to Pi starvation-induced protein GB:BAA06151 from [Nicotiana tabacum] (evalue: 8.5e-31, score=130.6) genbank/nr: gi|7489176|pir||T03677 pit2 protein (clone pAL141), Pi starvation induced - common tobacco gi|676884|dbj|BAA06151.1| The expression is induced by Pi starvation. [Nicotiana tabacum] gi|1094819|prf||2106387C Al-induced protein (evalue: 2e-36, score=154.5)	1.43	0.01
	TRANSCRIPTION FACTOR		
SGN-U213896	arabidopsis/peptide: At3g01470.1 68410.m00060 homeobox-leucine zipper protein HAT5 (HD-ZIP protein 5) (HD-ZIP protein ATHB-1) identical to homeobox-leucine zipper protein HAT5 (HD-ZIP protein 5) (HD-ZIP protein ATHB-1) GB:Q02283 [Arabidopsis thaliana] (evalue: 5.9e-38, score=155.6) genbank/nr: gi|15148918|gb|AAK84886.1| homeodomain leucine zipper protein	1.44	0.09

… continued …

TABLE 1 Cont.

	HDZ2 [Phaseolus vulgaris] (evalue: 4.2e-78, score=294.3)		
SGN-U214021	arabidopsis/peptide: At1g69780.1 68408.m07365 homeobox-leucine zipper protein ATHB-13 (HD-Zip transcription factor Athb-13) identical to homeobox gene 13 protein (GP:12325190) [Arabidopsis thaliana] (evalue: 7.4e-99, score=357.8) genbank/nr: gi|48057565|gb|AAT39931.1| putative HD-zip protein [Solanum demissum] (evalue: 4e-174, score=613.2)	1.4	0.06
SGN-U214635	arabidopsis/peptide: At2g35940.2 68409.m05699 homeodomain protein contains 'Homeobox' domain signature, Prosite:PS00027 (evalue: 3.4e-83, score=306.2) genbank/nr: gi|31323447|gb|AAP47025.1| bell-like homeodomain protein 2 [Lycopersicon esculentum] (evalue: 0, score=729.6)	2.54	0.07
SGN-U214896	arabidopsis/peptide: At2g41900.1 68409.m04689 CCCH-type zinc finger protein -related also an ankyrin-repeat protein (evalue: 0, score=667.5) genbank/nr: gi|28273376|gb|AAO38462.1| unknown prot [Oryza sativa (japonica cultivar-group)] (evalue: 0, score=674.5)	2	0.05
SGN-U215777	arabidopsis/peptide: At3g55770.1 68410.m05721 transcription factor L2 (evalue: 8.9e-93, score=337) genbank/nr: gi|7489184|pir||T03400 probable transcription factor SF3 - common tobacco gi|1841464|emb|CAA71891.1| LIM-domain SF3 protein [Nicotiana tabacum] gi|5932420|gb|AAD56951.1| LIM domain protein WLIM2 [Nicotiana tabacum] (evalue: 2e-103, score=377.9)	1.52	0.01
SGN-U216582	arabidopsis/peptide: At4g16420.1 68411.m02261 transcriptional adaptor like protein (evalue: 6.5e-23, score=104.8) genbank/nr: gi|18414653|ref|NP_567495.1| transcriptional adaptor like protein [Arabidopsis thaliana] gi|13591700|gb|AAK31320.1| transcriptional adaptor ADA2b [Arabidopsis thaliana] gi|15215640|gb|AAK91365.1| AT4g16420/dl4235c [Arabidopsis thaliana] gi|23505981|gb|AAN28850.1| At4g16420/dl4235c [Arabidopsis thaliana] (evalue: 2.4e-21, score=104.8)	2.56	0.02
SGN-U216849	arabidopsis/peptide: At5g62000.3 68418.m07784 transcriptional factor B3 family protein / auxin-responsive factor, putative (ARF1) contains Pfam profile: PF02362 B3 DNA binding domain; identical to cDNA ARF1 (auxin response factor) binding protein GI:2245393 (evalue: 4e-167, score=565.5) genbank/nr: gi|30027167|gb|AAP06759.1| auxin response factor-like protein [Mangifera indica] (evalue: 2e-169, score=580.5)	1.73	0.04
SGN-U217929	arabidopsis/peptide: At3g60800.1 68410.m06284 DHHC-type zinc finger domain-containing protein contains DHHC zinc finger domain PF01529 (evalue: 2.2e-72, score=268.9) genbank/nr: gi|22331887|ref|NP_191639.2| DHHC-type zinc finger domain-containing protein [Arabidopsis thaliana] gi|17979303|gb|AAL49877.1| unknown protein [Arabidopsis thaliana] gi|20466005|gb|AAM20224.1| unknown protein [Arabidopsis thaliana] (evalue: 7.9e-71, score=268.9)	1.57	0.09

… continued …

TABLE 1 Cont.

SGN-U219383	arabidopsis/peptide: At1g10200.1 68408.m01037 transcription factor - related similar to transcription factor SF3 (pir|IS37656); similar to ESTs gb|T42207, gb|N37716, and emb|Z17491 (evalue: 7.2e-74, score=274.2) genbank/nr: gi|5932418|gb|AAD56950.1| LIM domain protein PLIM1 [Nicotiana tabacum] (evalue: 7.7e-75, score=282.7)	1.7	0.06
SGN-U221901	arabidopsis/peptide: At1g26580.1 68408.m02934 expressed protein similar to putative MYB family transcription factor GB:AAD17429 GI:4335752 from [Arabidopsis thaliana] (evalue: 3.2e-11, score=66.24) genbank/nr: gi|15222736|ref|NP_173980.1| expressed protein [Arabidopsis thaliana] gi|25518777|pir||H86392 hypothetical protein T1K7.5 - Arabidopsis thaliana gi|9797742|gb|AAF98560.1| Contains similarity to a putative MYB family transcription factor gene T4M8.10 gi|4335752 from Arabidopsis thaliana BAC T4M8 gb|AC006284 and contains a Myb-like DNA-binding PF|00249 domain. ESTs gb|T75914, gb|T45901 come from this gene (evalue: 1.2e-09, score=66.24)	1.63	0.09
SGN-U222328	arabidopsis/peptide: At5g13790.1 68412.m01484 floral homeotic protein, AGL15 (evalue: 8.2e-51, score=198) genbank/nr: gi|34452081|gb|AAQ72497.1| MADS-box protein 9 [Petunia x hybrida] (evalue: 7e-107, score=389.4)	1.59	0.06
SGN-U222814	arabidopsis/peptide: At3g46640.1 68410.m04691 myb family transcription factor contains Pfam profile: PF00249 myb-like DNA-binding domain (evalue: 1.5e-16, score=83.57) genbank/nr: gi|15232597|ref|NP_190248.1| myb family transcription factor [Arabidopsis thaliana] gi|11357354|pir||T45601 hypothetical protein F12A12.160 - Arabidopsis thaliana gi|6523067|emb|CAB62334.1| putative protein [Arabidopsis thaliana] gi|30102630|gb|AAP21233.1| At3g46640 [Arabidopsis thaliana] (evalue: 5.4e-15, score=83.57)	1.99	0.09
SGN-U223527	arabidopsis/peptide: At1g77980.1 68414.m09087 MADS-box family protein MADS-box protein AGL66 (evalue: 1.5e-13, score=72.4) genbank/nr: gi|42408790|dbj|BAD10025.1| putative MADS-box protein [Oryza sativa (japonica cultivar-group)] >gi|42408843|dbj|BAD10102.1| putative MADS-box protein [Oryza sativa (japonica cultivar-group)] (evalue: 9.5e-15, score=81.26)	1.54	0.05
SGN-U228962	arabidopsis/peptide: At2g38250.1 68409.m04249 GT-1-related transcription factor (evalue: 9.9e-25, score=110.5) genbank/nr: gi|38344145|emb|CAD41865.2| OSJNBa0041A02.12 [Oryza sativa (japonica cultivar-group)] (evalue: 6.7e-38, score=159.5)	2	0.03
SGN-U230858	arabidopsis/peptide: At2g40620.1 68409.m04539 bZip DNA binding protein identical to b-Zip DNA binding protein GI:2246376 from [Arabidopsis thaliana]; contains a bZIP transcription factor basic domain signature (PDOC00036) (evalue: 2.3e-31, score=132.5) genbank/nr: gi|15226727|ref|NP_181594.1| bZip DNA binding protein [Arabidopsis thaliana] gi|25408715|pir||G84831 probable bZIP transcription factor [imported] - Arabidopsis thaliana gi|2651296|gb|AAB87576.1| putative bZIP transcription factor [Arabidopsis thaliana] gi|18377632|gb|AAL66966.1| putative bZIP transcription factor [Arabidopsis thaliana]	1.42	0

… continued …

TABLE 1 Cont.

	gi|20465783|gb|AAM20380.1| putative bZIP transcription factor [Arabidopsis thaliana] (evalue: 8.3e-30, score=132.5)		
SGN-U231468	arabidopsis/peptide: At3g60800.1 68410.m06284 DHHC-type zinc finger domain-containing protein contains DHHC zinc finger domain PF01529 (evalue: 1e-36, score=149.8) genbank/nr: gi|11358343|pir||T47891 hypothetical protein T4C21.210 - Arabidopsis thaliana gi|7329690|emb|CAB82684.1| putative protein [Arabidopsis thaliana] (evalue: 3.4e-35, score=149.8)	1.52	0.05
	TRANSPORT		
SGN-U214683	arabidopsis/peptide: At5g62670.1 68412.m07112 ATPase, plasma membrane-type (proton pump), putative strong similarity to P-type H(+)-transporting ATPase from Nicotiana plumbaginifolia [SP|Q08435, SP|Q08436], Lycopersicon esculentum [GI:5901757, SP|P22180], Solanum tuberosum [GI:435003]; contains InterPro accession IPR001757: ATPase, E1-E2 type (evalue: 0, score=710.3) genbank/nr: gi|25290692|pir||T52412 H+-exporting ATPase (EC 3.6.3.6) plasma membrane isoform LHA2 [imported] - tomato gi|5901757|gb|AAD55399.1| plasma membrane H+-ATPase isoform LHA2 [Lycopersicon esculentum] gi|9789539|gb|AAF98344.1| plasma membrane H+-ATPase [Lycopersicon esculentum] (evalue: 0, score=760)	1.58	0
SGN-U215141	arabidopsis/peptide: At4g09650.1 68411.m01429 H+-transporting ATP synthase-related protein H+-transporting ATP synthase (EC 3.6.1.34) delta chain precursor, chloroplast - Nicotiana tabacum,PIR2:S26198 (evalue: 5.4e-68, score=254.6) genbank/nr: gi|416681|sp|P32980|ATPD_TOBAC ATP synthase delta chain, chloroplast precursor gi|280404|pir||S26198 H+-transporting two-sector ATPase (EC 3.6.3.14) delta chain precursor, chloroplast - common tobacco gi|19787|emb|CAA45153.1| chloroplast ATP synthase (delta subunit) [Nicotiana tabacum] (evalue: 9e-102, score=372.1)	1.53	0.01
SGN-U216732	arabidopsis/peptide: At5g09260.1 68412.m00988 hypothetical protein SNF7 protein - Saccharomyces cerevisiae, PIR:S52590 (evalue: 7.3e-43, score=170.2) genbank/nr: gi|15242368|ref|NP_196488.1| hypothetical protein [Arabidopsis thaliana] gi|9955513|emb|CAC05452.1| putative protein [Arabidopsis thaliana] gi|34365667|gb|AAQ65145.1| At5g09260 [Arabidopsis thaliana] (evalue: 2.5e-41, score=170.2)	1.47	0
SGN-U217510	arabidopsis/peptide: At1g14360.1 68408.m01539 expressed protein (evalue: 7.4e-62, score=232.6) genbank/nr: gi|48209877|gb|AAT40483.1| putative UDP-galactose transporter [Solanum demissum] (evalue: 5e-65, score=248.1)	1.52	0.02
SGN-U225750	arabidopsis/peptide: At4g35300.1 68411.m05407 transporter - related low similarity to hexose transporter [Solanum tuberosum] GI:8347246; contains Pfam profile PF00083: major facilitator superfamily protein (evalue: 4.1e-44, score=174.9) genbank/nr: gi|26986186|emb|CAD58958.1| hexose transporter [Hordeum vulgare subsp. vulgare] (evalue: 7.1e-45, score=182.6)	1.87	0

… continued …

TABLE 1 Cont.

	UNKNOWN		
SGN-U213029	arabidopsis/peptide: At3g17860.1 68410.m02052 expressed protein (evalue: 1.2e-26, score=117.1) genbank/nr: gi|39652276|dbj|BAD04851.1| hypothetical protein [Solanum tuberosum] (evalue: 2.1e-25, score=118.2)	1.4	0.09
SGN-U213465	arabidopsis/peptide: At1g69230.2 68414.m07930 expressed protein (evalue: 1.3e-31, score=132.9) genbank/nr: gi|25518436|pir||C86390 hypothetical protein T1K7.26 - Arabidopsis thaliana gi|9797761|gb|AAF98579.1| Contains similarity to PIR7A protein from Oryza sativa gb|Z34271 and contains an alpha/beta hydrolase fold PF|00561. [Arabidopsis thaliana] (evalue: 2.1e-32, score=140.6)	1.74	0.03
SGN-U213550	arabidopsis/peptide: At4g11220.1 68411.m01643 expressed protein 24 kDa seed maturation protein - Glycine max,PID:g4102690 (evalue: 1.3e-82, score=303.5) genbank/nr: gi|34909318|ref|NP_916006.1| OSJNBb0021A09.5 [Oryza sativa (japonica cultivar-group)] gi|20160502|dbj|BAB89453.1| OSJNBb0021A09.5 [Oryza sativa (japonica cultivar-group)] (evalue: 8.7e-83, score=309.3)	1.46	0.07
SGN-U214640	arabidopsis/peptide: At1g20580.1 68408.m02319 expressed protein (evalue: 4.7e-50, score=194.5) genbank/nr: gi|18394883|ref|NP_564119.1| expressed protein [Arabidopsis thaliana] gi|8886923|gb|AAF80609.1| F2D10.7 [Arabidopsis thaliana] gi|15724292|gb|AAL06539.1| At1g20580/F2D10_6 [Arabidopsis thaliana] gi|20334748|gb|AAM16235.1| At1g20580/F2D10_6 [Arabidopsis thaliana] (evalue: 1.7e-48, score=194.5)	1.48	0.09
SGN-U214730	arabidopsis/peptide: At4g04955.1 68411.m00631 expressed protein (evalue: 0, score=691) genbank/nr: gi|18412757|ref|NP_567276.1| expressed protein [Arabidopsis thaliana] gi|15028089|gb|AAK76575.1| unknown protein [Arabidopsis thaliana] gi|21281139|gb|AAM44996.1| unknown protein [Arabidopsis thaliana] (evalue: 0, score=691)	2.13	0.06
SGN-U214813	arabidopsis/peptide: At5g23530.1 68412.m02530 expressed protein contains similarity to PrMC3 [Pinus radiata] GI:5487873 (evalue: 1e-112, score=403.3) genbank/nr: gi|15237783|ref|NP_197744.1| expressed protein [Arabidopsis thaliana] gi|8809707|dbj|BAA97248.1| contains similarity to unknown protein~gb|AAF27018.1~gene_id:MQM1.21 [Arabidopsis thaliana] (evalue: 5e-111, score=403.3)	2.05	0.09
SGN-U215491	arabidopsis/peptide: At1g55160.1 68408.m05774 expressed protein (evalue: 5.9e-25, score=110.5) genbank/nr: gi|18405239|ref|NP_564677.1| expressed protein [Arabidopsis thaliana] gi|25405797|pir||C96593 unknown protein, 99945-98618 [imported] - Arabidopsis thaliana gi|12321579|gb|AAG50842.1| unknown protein [Arabidopsis thaliana] gi|12323174|gb|AAG51570.1| unknown protein; 99945-98618 [Arabidopsis thaliana] gi|13937163|gb|AAK50075.1| At1g55160/T7N22.11 [Arabidopsis thaliana] gi|22137148|gb|AAM91419.1| At1g55160/T7N22.11 [Arabidopsis thaliana] (evalue: 1.9e-23, score=110.5)	2.15	0.01

… continued …

TABLE 1 Cont.

SGN-U215613	arabidopsis/peptide: At1g02475.1 68408.m00181 expressed protein (evalue: 2.8e-66, score=249.2) genbank/nr: gi|18378973|ref|NP_563656.1| expressed protein [Arabidopsis thaliana] gi|13878059|gb|AAK44107.1| unknown protein [Arabidopsis thaliana] gi|17104651|gb|AAL34214.1| unknown protein [Arabidopsis thaliana] (evalue: 1.1e-64, score=249.2)	2.2	0.03
SGN-U215870	arabidopsis/peptide: At1g03250.1 68408.m00271 expressed protein EST gb|N96383 comes from this gene (evalue: 2.8e-73, score=272.3) genbank/nr: gi|18379075|ref|NP_563680.1| expressed protein [Arabidopsis thaliana] gi|21593601|gb|AAM65568.1| unknown [Arabidopsis thaliana] gi|22022560|gb|AAM83237.1| At1g03250/F15K9_13 [Arabidopsis thaliana] gi|23308263|gb|AAN18101.1| At1g03250/F15K9_13 [Arabidopsis thaliana] (evalue: 1e-71, score=272.3)	1.7	0.01
SGN-U216025	arabidopsis/peptide: At5g19300.1 68412.m02105 hypothetical protein predicted proteins, H. sapiens, D. melanogaster and others (evalue: 2e-120, score=429.5) genbank/nr: gi|42567956|ref|NP_197431.2| expressed protein [Arabidopsis thaliana] >gi|45825145|gb|AAS77480.1| At5g19300 [Arabidopsis thaliana] (evalue: 8e-119, score=429.5)	1.82	0.02
SGN-U216096	No hits found	1.71	0.04
SGN-U216110	arabidopsis/peptide: At5g51010.1 68412.m05714 expressed protein (evalue: 2.9e-38, score=156) genbank/nr: gi|18423226|ref|NP_568749.1| expressed protein [Arabidopsis thaliana] gi|9758248|dbj|BAB08747.1| gene_id:K3K7.19~unknown protein [Arabidopsis thaliana] gi|15292669|gb|AAK92703.1| unknown protein [Arabidopsis thaliana] gi|19310697|gb|AAL85079.1| unknown protein [Arabidopsis thaliana] gi|21555410|gb|AAM63852.1| unknown [Arabidopsis thaliana] (evalue: 1.1e-36, score=156)	1.48	0.06
SGN-U216215	arabidopsis/peptide: At1g07080.1 68408.m00686 expressed protein (evalue: 1.6e-74, score=276.6) genbank/nr: gi|25406953|pir||F86205 hypothetical protein [imported] - Arabidopsis thaliana gi|8954038|gb|AAF82212.1| Contains similarity to an unknown protein F7A7_100 gi|7327817 from Arabidopsis thaliana BAC F7A7 gb|AL161946. ESTs gb|N65842, gb|F19836 and gb|AI993679 come from this gene (evalue: 6.2e-73, score=276.6)	1.69	0.02
SGN-U217127	arabidopsis/peptide: At5g04080.1 68412.m00365 expressed protein (evalue: 2.8e-12, score=68.94) genbank/nr: gi|30680382|ref|NP_196028.2| expressed protein [Arabidopsis thaliana] (evalue: 9.7e-11, score=68.94)	1.63	0.06
SGN-U217198	arabidopsis/peptide: At5g08050.1 68412.m00872 expressed protein predicted protein, Arabidopsis thaliana (evalue: 8.6e-33, score=136.7) genbank/nr: gi|17978981|gb|AAL47451.1| AT5g08050/F13G24_250 [Arabidopsis thaliana] gi|33589718|gb|AAQ22625.1| At5g08050/F13G24_250 [Arabidopsis thaliana] (evalue: 2.9e-31, score=136.7)	1.77	0.02

… continued …

TABLE 1 Cont.

SGN-U217288	arabidopsis/peptide: At2g03420.1 68409.m00267 expressed protein (evalue: 4.7e-46, score=181.4) genbank/nr: gi|18395549|ref|NP_565301.1| expressed protein [Arabidopsis thaliana] gi|20197739|gb|AAD17434.2| hypothetical protein [Arabidopsis thaliana] gi|26450217|dbj|BAC42227.1| unknown protein [Arabidopsis thaliana] gi|28827508|gb|AAO50598.1| unknown protein [Arabidopsis thaliana] (evalue: 1.7e-44, score=181.4)	1.77	0.1
SGN-U217312	arabidopsis/peptide: At1g11760.1 68408.m01217 expressed protein (evalue: 1.6e-44, score=176.4) genbank/nr: gi|28466841|gb|AAO44029.1| At1g11760 [Arabidopsis thaliana] (evalue: 5.2e-47, score=189.9)	1.48	0.02
SGN-U217758	arabidopsis/peptide: At2g35880.1 68409.m03986 expressed protein (evalue: 2.2e-55, score=213.8) genbank/nr: gi|18403980|ref|NP_565829.1| expressed protein [Arabidopsis thaliana] gi|16209720|gb|AAL14415.1| At2g35880/F11F19.21 [Arabidopsis thaliana] gi|20197995|gb|AAD21469.2| expressed protein [Arabidopsis thaliana] gi|22655282|gb|AAM98231.1| unknown protein [Arabidopsis thaliana] (evalue: 8.6e-54, score=213.8)	1.63	0.07
SGN-U217844	arabidopsis/peptide: At3g07568.1 68410.m00823 expressed protein (evalue: 9e-09, score=57) genbank/nr: gi|30680359|ref|NP_850535.1| expressed protein [Arabidopsis thaliana] gi|17065402|gb|AAL32855.1| Unknown protein [Arabidopsis thaliana] gi|20148611|gb|AAM10196.1| unknown protein [Arabidopsis thaliana] gi|21554046|gb|AAM63127.1| unknown [Arabidopsis thaliana] (evalue: 3.1e-07, score=57)	1.69	0.01
SGN-U218009	arabidopsis/peptide: At5g19855.1 68412.m02163 expressed protein (evalue: 2.5e-60, score=229.2) genbank/nr: gi|18420050|ref|NP_568382.1| expressed protein [Arabidopsis thaliana] gi|21593746|gb|AAM65713.1| unknown [Arabidopsis thaliana] gi|28393243|gb|AAO42050.1| unknown protein [Arabidopsis thaliana] gi|28827676|gb|AAO50682.1| unknown protein [Arabidopsis thaliana] (evalue: 9.6e-59, score=229.2)	1.97	0.01
SGN-U218047	arabidopsis/peptide: At1g29700.1 68408.m03287 expressed protein (evalue: 2e-105, score=379.4) genbank/nr: gi|18397206|ref|NP_564334.1| expressed protein [Arabidopsis thaliana] gi|25403086|pir||D86420 unknown protein [imported] - Arabidopsis thaliana gi|12321412|gb|AAG50777.1| unknown protein [Arabidopsis thaliana] gi|12323515|gb|AAG51727.1| unknown protein; 129333-127623 [Arabidopsis thaliana] gi|14596083|gb|AAK68769.1| Unknown protein [Arabidopsis thaliana] gi|18377530|gb|AAL66931.1| unknown protein [Arabidopsis thaliana] (evalue: 7e-104, score=379.4)	1.59	0.04
SGN-U218081	arabidopsis/peptide: At2g39950.1 68409.m04448 expressed protein and genscan (evalue: 3e-46, score=181.4) genbank/nr: gi|15225598|ref|NP_181524.1| expressed protein [Arabidopsis thaliana] gi|25408647|pir||D84823 hypothetical protein At2g39950 [imported] - Arabidopsis thaliana gi|2088648|gb|AAB95280.1| hypothetical protein [Arabidopsis thaliana] (evalue: 1e-44, score=181.4)	1.68	0.09

… continued …

TABLE 1 Cont.

SGN-U218211	arabidopsis/peptide: At2g33845.1 68409.m03754 expressed protein (evalue: 1.5e-39, score=159.8) genbank/nr: gi|18403397|ref|NP_565774.1| expressed protein [Arabidopsis thaliana] gi|20198312|gb|AAM15519.1| Expressed protein [Arabidopsis thaliana] gi|21592737|gb|AAM64686.1| unknown [Arabidopsis thaliana] gi|22530954|gb|AAM96981.1| expressed protein [Arabidopsis thaliana] gi|23198430|gb|AAN15742.1| expressed protein [Arabidopsis thaliana] (evalue: 5.5e-38, score=159.8)	1.61	0.01
SGN-U218673	arabidopsis/peptide: At4g00370.1 68411.m00049 expressed protein (evalue: 0, score=640.2) genbank/nr: gi|30678625|ref|NP_567175.2| expressed protein [Arabidopsis thaliana] gi|26451814|dbj|BAC43000.1| unknown protein [Arabidopsis thaliana] gi|32306495|gb|AAP78931.1| At4g00370 [Arabidopsis thaliana] (evalue: 0, score=640.2)	1.57	0.02
SGN-U218850	arabidopsis/peptide: At1g62310.1 68408.m06446 expressed protein (evalue: 1.4e-63, score=147.1) genbank/nr: gi|15220761|ref|NP_176421.1| expressed protein [Arabidopsis thaliana] (evalue: 5.7e-62, score=147.1)	1.8	0.04
SGN-U219158	arabidopsis/peptide: At1g02070.1 68408.m00117 expressed protein (evalue: 1.2e-10, score=63.54) genbank/nr: gi|46806700|dbj|BAD17770.1| hypothetical protein [Oryza sativa (japonica cultivar-group)] >gi|46806728|dbj|BAD17778.1| hypothetical protein [Oryza sativa (japonica cultivar-group)] (evalue: 3.7e-16, score=87.04)	3.09	0.03
SGN-U219500	arabidopsis/peptide: At2g31560.2 68415.m03856 expressed protein (evalue: 5.1e-48, score=188.3) genbank/nr: gi|30684991|ref|NP_850169.1| expressed protein [Arabidopsis thaliana] gi|17528942|gb|AAL38681.1| unknown protein [Arabidopsis thaliana] (evalue: 1.9e-46, score=188.3)	1.78	0.01
SGN-U219563	arabidopsis/peptide: At3g10260.2 68410.m06636 expressed protein similar to unknown protein GB:AAC62889 [Arabidopsis thaliana] (evalue: 2.3e-77, score=285.8) genbank/nr: gi|30681227|ref|NP_850552.1| expressed protein [Arabidopsis thaliana] (evalue: 8.8e-76, score=285.8)	1.56	0.03
SGN-U220069	arabidopsis/peptide: At3g56880.1 68410.m05844 expressed protein predicted protein At2g41010 - Arabidopsis thaliana, EMBL:AC004261 (evalue: 1.7e-20, score=97.06) genbank/nr: gi|15230149|ref|NP_191247.1| expressed protein [Arabidopsis thaliana] gi|11289614|pir||T51276 hypothetical protein T8M16_210 - Arabidopsis thaliana gi|9663007|emb|CAC00751.1| putative protein [Arabidopsis thaliana] gi|15028355|gb|AAK76654.1| unknown protein [Arabidopsis thaliana] gi|24030447|gb|AAN41377.1| unknown protein [Arabidopsis thaliana] (evalue: 6.4e-19, score=97.06)	1.43	0.05
SGN-U222203	arabidopsis/peptide: At2g27830.1 68409.m03055 expressed protein (evalue: 2.4e-37, score=152.5) genbank/nr: gi|10279678|emb|CAC09928.1| hypothetical protein [Catharanthus roseus] (evalue: 3.8e-47, score=190.3)	1.59	0.01
SGN-U222620	No hits found	3.76	0.04

… continued …

TABLE 1 Cont.

SGN-U222946	No hits found	1.51	0.1
SGN-U222946	No hits found	1.54	0.03
SGN-U223214	arabidopsis/peptide: At3g15050.1 68410.m01708 expressed protein similar to SF16 protein GB:CAA52782 [Helianthus annuus] (evalue: 9.5e-56, score=214.5) genbank/nr: gi|15232474|ref|NP_188123.1| expressed protein [Arabidopsis thaliana] gi|8777488|dbj|BAA97068.1| contains similarity to SF16 protein~gene_id:K15M2.19 [Arabidopsis thaliana] (evalue: 3.7e-54, score=214.5)	1.68	0.09
SGN-U223235	arabidopsis/peptide: At2g02050.1 68409.m00120 expressed protein (evalue: 5.2e-41, score=163.7) genbank/nr: gi|18395290|ref|NP_565280.1| expressed protein [Arabidopsis thaliana] gi|25410957|pir||D84432 hypothetical protein At2g02050 [imported] - Arabidopsis thaliana gi|4406787|gb|AAD20097.1| expressed protein [Arabidopsis thaliana] gi|11692816|gb|AAG40011.1| At2g02050 [Arabidopsis thaliana] gi|11908122|gb|AAG41490.1| unknown protein [Arabidopsis thaliana] gi|12642932|gb|AAK00408.1| unknown protein [Arabidopsis thaliana] gi|15529288|gb|AAK97738.1| At2g02050/F14H20.12 [Arabidopsis thaliana] gi|21593473|gb|AAM65440.1| unknown [Arabidopsis thaliana] gi|23505757|gb|AAN28738.1| At2g02050/F14H20.12 [Arabidopsis thaliana] (evalue: 1.7e-39, score=163.7)	1.64	0.04
SGN-U223256	arabidopsis/peptide: At5g59080.1 68412.m06698 expressed protein (evalue: 4.2e-11, score=65.47) genbank/nr: gi|15237814|ref|NP_200716.1| expressed protein [Arabidopsis thaliana] gi|10177635|dbj|BAB10783.1| emb|CAB82975.1~gene_id:K18B18.8~similar to unknown protein [Arabidopsis thaliana] gi|17380960|gb|AAL36292.1| unknown protein [Arabidopsis thaliana] gi|20465393|gb|AAM20121.1| unknown protein [Arabidopsis thaliana] (evalue: 1.6e-09, score=65.47)	1.53	0.06
SGN-U225377	No hits found	1.83	0.01
SGN-U228094	No hits found	2.17	0.06
SGN-U230225	arabidopsis/peptide: At5g67480.1 68412.m07705 expressed protein strong similarity to unknown protein (pir||T04718) (evalue: 2e-43, score=172.2) genbank/nr: gi|15240763|ref|NP_201549.1| expressed protein [Arabidopsis thaliana] gi|9757869|dbj|BAB08456.1| gene_id:K9I9.4~pir||T04718~strong similarity to unknown protein [Arabidopsis thaliana] gi|15529178|gb|AAK97683.1| AT5g67480/K9I9_4 [Arabidopsis thaliana] gi|17386120|gb|AAL38606.1| AT5g67480/K9I9_4 [Arabidopsis thaliana] (evalue: 6.8e-42, score=172.2)	1.53	0.01

… continued …

TABLE 1 Cont.

SGN-U231560	arabidopsis/peptide: At4g27540.1 68411.m03603 expressed protein (evalue: 6.7e-14, score=73.94) genbank/nr: gi|18417012|ref|NP_567776.1| expressed protein [Arabidopsis thaliana] gi|21536961|gb|AAM61302.1| unknown [Arabidopsis thaliana] (evalue: 2.2e-12, score=73.94)	1.98	0.03
SGN-U232066	No hits found	1.83	0.09
SGN-U232089	No hits found	2.28	0.02
SGN-U232169	No hits found	2.29	0.1
SGN-U234375	No hits found	1.47	0
SGN-U234755	No hits found	2.03	0.08
SGN-U236156	No hits found	1.74	0.04
SGN-U237464	No hits found	1.47	0.05
SGN-U240428	No hits found	1.49	0.1
SGN-U240837	No hits found	2.04	0.01
SGN-U241296	No hits found	2.71	0.05

… continued …

TABLE 1 Cont.

ID	Gene Annotation	Average of ratios	Pvalue
	DOWN-REGULATED GENES		
	CELL STRUCTURE		
SGN-U213235	arabidopsis/peptide: At5g65360.1 68412.m07436 histone H3 identical to histone H3 from Zea mays SP|P05203, Medicago sativa GI:166384, Encephalartos altensteinii SP|P08903, Pisum sativum SP|P02300; contains Pfam profile PF00125 Core histone H2A/H2B/H3/H4 (evalue: 2.9e-56, score=214.9) genbank/nr: gi|15232146|ref|NP_189372.1| histone H3 [Arabidopsis thaliana] gi|15238433|ref|NP_201339.1| histone H3 [Arabidopsis thaliana] H3 histone [Nicotiana tabacum] gi|27764990|gb|AAO23616.1| At5g10400 [Arabidopsis thaliana] gi|27808628|gb|AAO24594.1| At1g09200 [Arabidopsis thaliana] gi|28973783|gb|AAO64207.1| putative histone H3 [Arabidopsis thaliana] gi|29824185|gb|AAP04053.1| putative histone H3 [Arabidopsis thaliana] gi|38345293|emb|CAE02917.2| OSJNBb0108J11.9 [Oryza sativa (japonica cultivar-group)] gi|225459|prf||1303352A histone H3 gi|225839|prf||1314298B histone H3 (evalue: 1e-54, score=214.9)	0.58	0.06
SGN-U215583	arabidopsis/peptide: At1g07790.1 68408.m00763 histone H2B, putative strong similarity to histone H2B Arabidopsis thaliana GI:2407802, Gossypium hirsutum SP|O22582, Lycopersicon esculentum GI:3021489, Capsicum annuum SP|O49118; contains Pfam profile PF00125 Core histone H2A/H2B/H3/H4 (evalue: 2e-44, score=175.6) genbank/nr: gi|7387727|sp|O49118|H2B_CAPAN Histone H2B (CaH2B) gi|7439757|pir||T08063 histone H2B - pepper gi|2746719|gb|AAB94923.1| histone H2B [Capsicum annuum] (evalue: 5.4e-43, score=176)	0.6	0.01
SGN-U214148	arabidopsis/peptide: At2g30620.1 68409.m03380 histone H1 (evalue: 1.1e-18, score=91.28) genbank/nr: gi|585241|sp|P37218|H1_LYCES HISTONE H1 gi|629668|pir||S45662 histone H1 - tomato gi|424100|gb|AAA50578.1| histone H1 (evalue: 6e-32, score=140.6)	0.6	0.01

… continued …

TABLE 1 Cont.

	CELL WALL		
SGN-U231117	arabidopsis/peptide: At5g15050.1 68412.m01629 N-acetylglucosaminyltransferase family (Core-2/I-Branching enzyme family) contains Pfam profile: PF02485 Core-2/I-Branching enzyme (evalue: 1.2e-25, score=112.8) genbank/nr: gi|15242199|ref|NP_197009.1| N-acetylglucosaminyltransferase family (Core-2/I-Branching enzyme family) [Arabidopsis thaliana] gi|11291967|pir||T51450 hypothetical protein F2G14_170 - Arabidopsis thaliana gi|9755672|emb|CAC01824.1| putative protein [Arabidopsis thaliana] gi|16209674|gb|AAL14395.1| AT5g15050/F2G14_170 [Arabidopsis thaliana] gi|21554320|gb|AAM63425.1| putative glycosylation enzyme [Arabidopsis thaliana] gi|21700835|gb|AAM70541.1| AT5g15050/F2G14_170 [Arabidopsis thaliana] (evalue: 3.8e-24, score=112.8)	0.38	0.09
SGN-U214672	arabidopsis/peptide: At2g45220.1 68409.m05085 pectinesterase family contains Pfam profile: PF01095 pectinesterase (evalue: 7e-174, score=607.4) genbank/nr: gi|7447381|pir||T10494 pectinesterase (EC 3.1.1.11) PECS-c2 - sweet orange gi|2098713|gb|AAB57671.1| pectinesterase [Citrus sinensis] (evalue: 3e-176, score=620.5)	0.47	0.02
	DEFENSE RESPONSE		
SGN-U220257	arabidopsis/peptide: At3g48090.1 68410.m04852 disease resistance protein (EDS1) identical to disease resistance protein/lipase homolog EDS1 GI:4454567; contains Pfam profile PF01764: Lipase (evalue: 8.7e-62, score=234.6) genbank/nr: gi|19110917|gb|AAL85347.1| EDS1-like protein [Nicotiana benthamiana] (evalue: 3e-158, score=560.5)	0.39	0.1
SGN-U223757	arabidopsis/peptide: At5g23960.1 68412.m02576 terpene synthase/cyclase family non-consensus TA donor splice site at exon 4 (evalue: 6.3e-60, score=227.3) genbank/nr: gi|4105137|gb|AAD02270.1| putative vetispiradiene synthase 5 [Solanum tuberosum] gi|5360687|dbj|BAA82109.1| vetispiradiene synthase [Solanum tuberosum] (evalue: 5e-140, score=498.4)	0.6	0.01

… continued …

TABLE 1 Cont.

	ENERGY PATHWAYS		
SGN-U215146	arabidopsis/peptide: At1g78570.1 68408.m08402 NAD-dependent epimerase/dehydratase family similar to dTDP-glucose 4,6-dehydratase from Aneurinibacillus thermoaerophilus GI:16357461, RmlB from Leptospira borgpetersenii GI:4234803; contains Pfam profile PF01370 NAD dependent epimerase/dehydratase family (evalue: 0, score=1177.5) genbank/nr: gi|15218420|ref|NP_177978.1| NAD-dependent epimerase/dehydratase family [Arabidopsis thaliana] gi|25406555|pir||C96814 hypothetical protein T30F21.10 [imported] - Arabidopsis thaliana gi|4836876|gb|AAD30579.1| Similar to dTDP-D-glucose 4,6-dehydratase [Arabidopsis thaliana] gi|14596091|gb|AAK68773.1| Similar to dTDP-D-glucose 4,6-dehydratase [Arabidopsis thaliana] gi|20148285|gb|AAM10033.1| similar to dTDP-D-glucose 4,6-dehydratase [Arabidopsis thaliana] (evalue: 0, score=1177.5)	0.49	0.02
SGN-U223571	arabidopsis/peptide: At1g56560.1 68408.m05963 alkaline/neutral invertase -related similar to alkaline/neutral invertase GI:9758657 from [Arabidopsis thaliana] (evalue: 3e-119, score=424.9) genbank/nr: gi|15223561|ref|NP_176049.1| alkaline/neutral invertase -related [Arabidopsis thaliana] gi|25354738|pir||C96607 probable invertase F25P12.99 [imported] - Arabidopsis thaliana gi|9954756|gb|AAG09107.1| Putative invertase [Arabidopsis thaliana] gi|21539565|gb|AAM53335.1| putative alkaline/neutral invertase [Arabidopsis thaliana] gi|30725448|gb|AAP37746.1| At1g56560 [Arabidopsis thaliana] (evalue: 1e-117, score=424.9)	0.55	0.02
	HORMONE RESPONSE		
SGN-U221653	arabidopsis/peptide: At1g74670.1 68408.m07941 GAST1-related protein similar to GAST1 protein precursor GB:P27057 [Lycopersicon esculentum] (induced by gibberellins, inhibited by ABA Plant J 1992 Mar;2(2):153-9) (evalue: 1.2e-31, score=132.9) genbank/nr: gi|121689|sp|P27057|GST1_LYCES GAST1 protein precursor gi|100217|pir||S22151 gibberellin-regulated protein GAST1 - tomato gi|19247|emb|CAA44807.1| gast1 [Lycopersicon esculentum] (evalue: 2.1e-36, score=153.7)	0.49	0.03
SGN-U222410	arabidopsis/peptide: At1g66340.1 68408.m06909 ethylene-response protein, ETR1 identical to GB:P49333 from [Arabidopsis thaliana] (Science 262 (5133), 539-544 (1993)) (evalue: 2.6e-46, score=181.8) genbank/nr: gi|4210924|gb|AAD12777.1| ethylene receptor homolog [Solanum tuberosum] (evalue: 4.8e-59, score=229.2)	0.6	0.06

… continued …

TABLE 1 Cont.

	LIPID METABOLISM		
SGN-U223765	arabidopsis/peptide: At5g08415.1 68412.m00922 lipoic acid synthase family similar to lipoic acid synthase from Arabidopsis thaliana [gi:3928758], from Mus musculus [gi:14669826] Pfam profile PF04055: radical SAM domain protein (evalue: 1.9e-69, score=258.8) genbank/nr: gi|18415808|ref|NP_568196.1| lipoic acid synthase family [Arabidopsis thaliana] gi|20373023|dbj|BAB91180.1| lipoic acid synthase [Arabidopsis thaliana] (evalue: 6.5e-68, score=258.8)	0.42	0.02
SGN-U214975	arabidopsis/peptide: At5g46290.1 68412.m05136 3-oxoacyl-[acyl-carrier-protein] synthase I precursor (beta-ketoacyl-acp synthase I) (KAS I) (sp|P52410) (evalue: 0, score=782.7) genbank/nr: gi|7433753|pir||T10061 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41) precursor, chloroplast - castor bean gi|294668|gb|AAA33873.1| beta-ketoacyl-ACP synthase (evalue: 0, score=793.5)	0.56	0.08
	METABOLISM		
SGN-U220719	arabidopsis/peptide: At3g54690.1 68410.m05597 sugar isomerase (SIS) domain-containing protein similar to SP|Q47334 Polysialic acid capsule expression protein kpsF {Escherichia coli}; contains Pfam profiles PF01380: sugar isomerase (SIS) domain, PF00571: CBS domain (evalue: 2.2e-89, score=325.5) genbank/nr: gi|15232565|ref|NP_191029.1| sugar isomerase (SIS) domain-containing protein [Arabidopsis thaliana] gi|11278593|pir||T47628 sugar-phosphate isomerase-like protein - Arabidopsis thaliana gi|7258373|emb|CAB77589.1| sugar-phosphate isomerase-like protein [Arabidopsis thaliana] (evalue: 8e-88, score=325.5)	0.55	0.06
	OTHERS		
SGN-U235658	putative membrane-associated salt-inducible protein [Arabidopsis thaliana]	0.33	0.02
SGN-U237426	hypothetical protein DDBDRAFT_0219654 [Dictyostelium discoideum	0.44	0.04
SGN-U215755	arabidopsis/peptide: At1g72610.1 68408.m07704 germin-like protein (AtGER1) identical to germin-like protein subfamily 3 member 1 SP|P94040; contains Pfam profile: PF01072 Germin family (evalue: 8.9e-74, score=273.5) genbank/nr: gi|18203684|sp|Q9ZRA4|ABPA_PRUPE Auxin-binding protein ABP19a precursor gi|4098517|gb|AAD00295.1| auxin-binding protein ABP19 [Prunus persica] (evalue: 3.5e-79, score=296.6)	0.44	0.03

… continued …

TABLE 1 Cont.

SGN-U215862	arabidopsis/peptide: At3g18890.1 68410.m02166 expressed protein similar to UV-B and ozone similarly regulated protein 1 UOS1 [Pisum sativum] GI:20339364 (evalue: 3e-122, score=436) genbank/nr: gi|21616072|emb|CAC87810.2| Tic62 protein [Pisum sativum] (evalue: 5e-127, score=457.2)	0.53	0.07
SGN-U236129	dopamine beta-monooxygenase [Arabidopsis thaliana]	0.55	0.06
SGN-U214185	arabidopsis/peptide: At3g27090.1 68410.m03103 gda-1 - related similar to gda-1 GB:CAA74993 from [Pisum sativum] (evalue: 4e-114, score=408.7) genbank/nr: gi|2369766|emb|CAA04664.1| hypothetical protein [Citrus x paradisi] (evalue: 2e-117, score=424.9)	0.57	0.09
	PHOTOSYNTHESIS		
SGN-U234089	chlorophyll a-b binding protein 3C-like [Solanum tuberosum]	0.33	0.03
SGN-U213489	arabidopsis/peptide: At4g27440.1 68411.m03591 protochlorophyllide reductase B (PCR B/POR B) identical to protochlorophyllide reductase B SP:P21218 from [Arabidopsis thaliana] (evalue: 2e-174, score=609.4) genbank/nr: gi|21068893|dbj|BAB93003.1| NADPH:protochlorophyllide oxidoreductase [Nicotiana tabacum] (evalue: 0, score=674.5)	0.34	0.03
SGN-U234089	chlorophyll a-b binding protein 3C-like [Solanum tuberosum]	0.38	0.06
SGN-U225521	arabidopsis/peptide: At5g38410.1 68412.m04170 ribulose bisphosphate carboxylase small chain 3b precursor (RuBisCO small subunit 3b) (sp|P10798) (evalue: 2.7e-74, score=275.8) genbank/nr: gi|132104|sp|P07180|RBS3_LYCES Ribulose bisphosphate carboxylase small chain 3A/3C, chloroplast precursor (RuBisCO small subunit 3A/3C) gi|68072|pir||RKTO3C ribulose-bisphosphate carboxylase (EC 4.1.1.39) small chain 3A precursor - tomato gi|19334|emb|CAA29402.1| ribulose 1,5-bisphosphate carboxylase/oxygenase [Lycopersicon esculentum] gi|19338|emb|CAA29404.1| ribulose 1,5-bisphosphate carboxylase/oxygenase [Lycopersicon esculentum] gi|170500|gb|AAA34190.1| ribulose-1,5-bisphophate carboxylase/ oxygenase small subunit (evalue: 7.7e-97, score=355.9)	0.43	0.07

… continued …

TABLE 1 Cont.

SGN-U225534	arabidopsis/peptide: At5g38410.1 68412.m04170 ribulose bisphosphate carboxylase small chain 3b precursor (RuBisCO small subunit 3b) (sp|P10798) (evalue: 3.5e-60, score=144.1) genbank/nr: gi|132095|sp|P07179|RBS2_LYCES Ribulose bisphosphate carboxylase small chain 2A, chloroplast precursor (RuBisCO small subunit 2A) (LESS 5) gi|68074|pir||RKTOS2 ribulose-bisphosphate carboxylase (EC 4.1.1.39) small chain 2 precursor - tomato gi|170498|gb|AAA34189.1| ribulose-1,5-bisphophate carboxylase/ oxygenase small subunit (EC 4.1.1.39) gi|4456641|emb|CAA29401.2| ribulose 1,5-bisphosphate carboxylase/oxygenase [Lycopersicon esculentum] (evalue: 1.2e-77, score=177.2)	0.43	0.01
SGN-U225538	arabidopsis/peptide: At5g38420.1 68412.m04171 ribulose bisphosphate carboxylase small chain 2b precursor (RuBisCO small subunit 2b) (sp|P10797) (evalue: 8e-73, score=270.4) genbank/nr: gi|132079|sp|P08706|RBS1_LYCES Ribulose bisphosphate carboxylase small chain 1, chloroplast precursor (RuBisCO small subunit 1) (LESS17) gi|68075|pir||RKTOS1 ribulose-bisphosphate carboxylase (EC 4.1.1.39) small chain 1 precursor - tomato gi|170496|gb|AAA34188.1| ribulose-1,5-bisphophate carboxylase/ oxygenase small subunit gi|295814|emb|CAA29400.1| ribulose 1,5-bisphosphate carboxylase/oxygenase [Lycopersicon esculentum] (evalue: 8.4e-95, score=348.6)	0.45	0.01
SGN-U212938	arabidopsis/peptide: At5g54270.1 68412.m06111 light-harvesting chlorophyll a/b binding protein, putative (evalue: 4e-137, score=484.6) genbank/nr: gi|115794|sp|P27489|CB23_LYCES Chlorophyll A-B binding protein 13, chloroplast precursor (LHCII type III CAB-13) gi|72748|pir||CDTO33 chlorophyll a/b-binding protein type III precursor (cab-13) - tomato gi|19277|emb|CAA42818.1| LHCII type III [Lycopersicon esculentum] (evalue: 8e-146, score=518.5)	0.56	0.08
M14444	Tomato chlorophyll a/b-binding protein gene Cab-3C, complete cds	0.58	0.01
SGN-U213031	arabidopsis/peptide: At1g20340.1 68408.m02290 plastocyanin similar to plastocyanin GI:1865683 from [Arabidopsis thaliana] (evalue: 3.6e-49, score=191.8) genbank/nr: gi|130271|sp|P17340|PLAS_LYCES Plastocyanin, chloroplast precursor gi|100238|pir||S05303 plastocyanin precursor - tomato gi|19300|emb|CAA32121.1| unnamed protein product [Lycopersicon esculentum] (evalue: 8.7e-60, score=232.3)	0.6	0.02
SGN-U234086	chlorophyll a/b-binding protein Cab-3C	0.6	0.01

… continued …

TABLE 1 Cont.

	PROTEIN BIOSYNTHESIS/DEGRADATION		
SGN-U237059	Eukaryotic translation initiation factor 3 subunit 10 (eIF-3 theta)	0.36	0.06
ID	Gene Annotation	Average of ratios	Pvalue
	PROTEINASE INHIBITOR		
SGN-U213363	genbank/nr: gi|3913937|sp|Q43710|IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi|629672|pir||S43338 proteinase inhibitor - tomato gi|405582|gb|AAA16881.1| proteinase inhibitor gi|462765|gb|AAC37397.1| auxin-induced proteinase inhibitor gi|742004|prf||2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.43	0.03
SGN-U213363	genbank/nr: gi|3913937|sp|Q43710|IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi|629672|pir||S43338 proteinase inhibitor - tomato gi|405582|gb|AAA16881.1| proteinase inhibitor gi|462765|gb|AAC37397.1| auxin-induced proteinase inhibitor gi|742004|prf||2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.43	0.05
SGN-U213363	genbank/nr: gi|3913937|sp|Q43710|IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi|629672|pir||S43338 proteinase inhibitor - tomato gi|405582|gb|AAA16881.1| proteinase inhibitor gi|462765|gb|AAC37397.1| auxin-induced proteinase inhibitor gi|742004|prf||2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.43	0
SGN-U213363	genbank/nr: gi|3913937|sp|Q43710|IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi|629672|pir||S43338 proteinase inhibitor - tomato gi|405582|gb|AAA16881.1| proteinase inhibitor gi|462765|gb|AAC37397.1| auxin-induced proteinase inhibitor gi|742004|prf||2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.46	0
SGN-U213363	genbank/nr: gi|3913937|sp|Q43710|IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi|629672|pir||S43338 proteinase inhibitor - tomato gi|405582|gb|AAA16881.1| proteinase inhibitor gi|462765|gb|AAC37397.1| auxin-induced proteinase inhibitor gi|742004|prf||2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.47	0.09
SGN-U213363	genbank/nr: gi|3913937|sp|Q43710|IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi|629672|pir||S43338 proteinase inhibitor - tomato gi|405582|gb|AAA16881.1| proteinase inhibitor gi|462765|gb|AAC37397.1| auxin-induced proteinase inhibitor gi|742004|prf||2008321A auxin-inducible protease	0.48	0.01

… continued …

TABLE 1 Cont.

	inhibitor (evalue: 3e-129, score=463)		
SGN-U213363	genbank/nr: gi|3913937|sp|Q43710|IP22_LYCES PROTEINASE INHIBITOR TYPE II TR8 PRECURSOR gi|629672|pir||S43338 proteinase inhibitor - tomato gi|405582|gb|AAA16881.1| proteinase inhibitor gi|462765|gb|AAC37397.1| auxin-induced proteinase inhibitor gi|742004|prf||2008321A auxin-inducible protease inhibitor (evalue: 3e-129, score=463)	0.49	0.06
	SIGNAL TRANSDUCTION		
SGN-U216491	arabidopsis/peptide: At5g47530.1 68412.m05290 auxin-induced protein, putative similar to auxin-induced protein AIR12 GI:11357190 [Arabidopsis thaliana] (evalue: 1e-112, score=403.7) genbank/nr: gi|13785207|emb|CAC37355.1| putative membrane protein [Solanum tuberosum] (evalue: 0, score=743)	0.54	0.04
	TRANSCRIPTION FACTORS		
SGN-U220511	arabidopsis /peptide: At3g14980.1 68410.m01700 PHD finger transcription factor, putative contains Pfam profile: PF00628 PHD-finger (evalue: 1.2e-44, score=176.8) genbank/nr: gi|8777481|dbj|BAA97061.1| gb|AAC80581.1~gene_id:K15M2.12~similar to unknown protein [Arabidopsis thaliana] (evalue: 4.4e-43, score=176.8)	0.41	0.02
SGN-U225750	arabidopsis/peptide: At5g67420.1 68412.m07697 lateral organ boundaries (LOB) domain protein 37 (LBD37) identical to LOB DOMAIN 37 [Arabidopsis thaliana] GI:17227170 (evalue: 3.7e-54, score=208.8) genbank/nr: gi|21593577|gb|AAM65544.1| unknown [Arabidopsis thaliana] (evalue: 1.1e-52, score=209.1)	0.43	0.04
SGN-U221689	arabidopsis/peptide: At5g67420.1 68412.m07697 lateral organ boundaries (LOB) domain protein 37 (LBD37) identical to LOB DOMAIN 37 [Arabidopsis thaliana] GI:17227170 (evalue: 3.7e-54, score=208.8) genbank/nr: gi|21593577|gb|AAM65544.1| unknown [Arabidopsis thaliana] (evalue: 1.1e-52, score=209.1)	0.44	0
SGN-U242047	ANAC057; transcription factor [Arabidopsis thaliana]	0.54	0.04
SGN-U221678	arabidopsis/peptide: At3g54810.2 68410.m06888 GATA zinc finger protein GATA transcription factor 3, Arabidopsis thaliana, Y13650 (evalue: 1.2e-14, score=77.03) genbank/nr: gi|37572451|dbj|BAC98495.1| AG-motif binding protein-5 [Nicotiana tabacum] (evalue: 3.8e-76, score=286.6)	0.55	0.03

… continued …

TABLE 1 Cont.

SGN-U225111	arabidopsis/peptide: At4g01250.1 68411.m00154 WRKY family transcription factor contains Pfam profile: PF03106 WRKY DNA -binding domain (evalue: 2.5e-47, score=186.4) genbank/nr: gi|27817201|gb|AAO23324.1| WRKY transcription factor 22 [Capsella rubella] gi|27817203|gb|AAO23325.1| WRKY transcription factor 22 [Capsella rubella] (evalue: 2e-46, score=188.7)	0.56	0.02
	TRANSPORT		
SGN-U236506	peptide transporter - like protein [Arabidopsis thaliana]	0.23	0.06
	UNKNOWN		
SGN-U230968	No hits found	0.4	0.03
SGN-U240735	No hits found	0.43	0.09
SGN-U227318	arabidopsis/peptide: At1g52870.2 68408.m05485 expressed protein (evalue: 7e-32, score=133.7) genbank/nr: gi|30695366|ref|NP_564615.3| expressed protein [Arabidopsis thaliana] gi|25405607|pir||H96569 unknown protein, 54928-56750 [imported] - Arabidopsis thaliana gi|12324641|gb|AAG52277.1| unknown protein; 54928-56750 [Arabidopsis thaliana] gi|14326545|gb|AAK60317.1| At1g52870/F14G24_14 [Arabidopsis thaliana] gi|25090145|gb|AAN72239.1| At1g52870/F14G24_14 [Arabidopsis thaliana] (evalue: 2.3e-30, score=133.7)	0.45	0.03
SGN-U218284	arabidopsis/peptide: At4g01150.1 68411.m00143 expressed protein (evalue: 9e-45, score=177.9) genbank/nr: gi|687677|gb|AAB00107.1| unknown (evalue: 2.7e-43, score=178.3)	0.46	0.03
SGN-U216503	arabidopsis/peptide: At5g47920.1 68412.m05334 expressed protein similar to unknown protein (emb|CAB67623.1) (evalue: 5.1e-33, score=138.7) genbank/nr: gi|15238843|ref|NP_199603.1| expressed protein [Arabidopsis thaliana] gi|10177928|dbj|BAB11339.1| emb|CAB67623.1~gene_id:MCA23.26~similar to unknown protein [Arabidopsis thaliana] gi|26449488|dbj|BAC41870.1| unknown protein [Arabidopsis thaliana] (evalue: 1.9e-31, score=138.7)	0.48	0.08
SGN-U225353	No hits found	0.54	0.01

… continued …

TABLE 1 Cont.

SGN-U220892	arabidopsis/peptide: At1g24480.1 68408.m02787 hypothetical protein similar to EST gb|AA394324 (evalue: 3.6e-88, score=322) genbank/nr: gi|25403245|pir||G86378 protein F21J9.14 [imported] - Arabidopsis thaliana gi|9743354|gb|AAF97978.1| F21J9.14 [Arabidopsis thaliana] (evalue: 6.2e-87, score=323.2)	0.55	0.06